

4748E2 File copy

HANDBOOK OF ANALYTICAL METHODS FOR ENVIRONMENTAL SAMPLES

VOLUME 1



Ontario

**Ministry
of the
Environment**

**Laboratory Services
and
Applied Research Branch**

TD
193
057
1983
V.1

Distribution Record

Due to the length of time required to produce this manual, it is possible that some changes in methods may not have been included in this handbook. A record of distribution has been established and will be maintained up to date so that any revisions of existing methods and additions of new methods may be distributed to users of this handbook. Please complete the form below and mail to:

**Handbook of Analytical Methods
Laboratory Services & Applied Research Branch
Water Quality Section
P.O. Box 213
Rexdale, Ontario, Canada
M9W 5L1**

(mail lower portion)

Address future revisions/additions of the "Handbook of Analytical Methods" to:

Surname Name _____

Initials _____

Organization: _____

Address: _____

City: _____

Province/State: _____

Country: _____

Postal Code: _____

Date

Signature

**HANDBOOK OF ANALYTICAL METHODS FOR
ENVIRONMENTAL SAMPLES**

DECEMBER, 1983

**ONTARIO MINISTRY OF THE ENVIRONMENT
LABORATORY SERVICES AND APPLIED RESEARCH BRANCH
REXDALE, ONTARIO, CANADA
M9W 5L1**

Disclaimer

The mention of trade names or commercial products in this manual is for illustration purposes and does not constitute endorsement or recommendation for use by the Laboratory Services and Applied Research Branch of the Ontario Ministry of the Environment.

FOREWORD

This "Handbook of Analytical Methods for Environmental Samples" was prepared by the scientists and technicians of the Laboratory Services and Applied Research Branch, Ontario Ministry of the Environment. The manual is the culmination of many years of effort. The analytical methods included in this manual are those used currently by the central laboratory and the regional laboratories of the ministry.

Thanks are expressed to the many people who participated in the production of this manual, from method development to documentation to proofreading.

Special acknowledgments are made to Serge Villard who managed the project; Ann Neary, Stan Wisz and W.B. Moody who co-ordinated the manual through its various stages; the Cartography and Drafting Section, Water Resources Branch who provided all the diagrams and to Marla Allen, Fran Hall and Sylvia Wilson who exhibited great patience in typing all the drafts, revisions and the final copy of this manual.

Gerard C. Ronan
Director

The President's Commission on the Assassination of President Kennedy has been established to investigate the assassination of President Kennedy. The Commission is composed of members from both the House and the Senate, and is headed by Chief Justice Warren. The Commission's report is expected to be released in the near future.

The Commission is currently conducting a thorough investigation of the assassination, and is expected to release its findings in the near future.

The Commission is currently conducting a thorough investigation of the assassination, and is expected to release its findings in the near future.

TABLE OF CONTENTS

Page

Forward	iii
Introduction	xi
Quality Control	xii

CHEMICAL AND PHYSICAL PARAMETERS

ACIDITY

A	Manual Fixed Endpoint Titration	AA 1
B	Automated Titration	AA 8

ALKALINITY

A	Semi-automated and Automated Fixed Endpoint: Potentiometric Titration	AB 1
B	Automated Inflection Point: Potentiometric Titration	AB 10

ALUMINUM

See Trace Metals

ANIONIC SURFACTANTS (MBAS)

A	Methylene Blue: Spectrophotometric	AC 1
---	------------------------------------	------

ARSENIC

A	Hydride Generation - Atomic Absorption	AD 1
---	--	------

ASBESTOS

A	Transmission Electron Microscopy	AE 1
---	----------------------------------	------

BARIUM

See Trace Metals

BERYLLIUM

See Trace Metals

CADMIUM

See Trace Metals

CALCIUM

A	Compleximetric Titration	CA 1
B	Automated Atomic Absorption	CA 8
	See also Trace Metals (5-3)	

CARBON

A	Dissolved Inorganic: Automated Colorimetry	CB 1
B	Dissolved Organic: UV Digestion - Automated Colorimetry	CB 13
C	Total and Inorganic: Infra-red Analysis	CB 20

CARBON DIOXIDE

A	Manual Titration	CC 1
B	Automated Titration	CC 7

TABLE OF CONTENTS

	Page
CHLORIDE	
A Automated Potentiometric Titration	CD 1
B Automated Colorimetry	CD 12
C Automated Ion Chromatography	CD 18
D Alkali Fusion - Semi Automated Colorimetry	CD 20
See also Trace Metals (5-3)	
CHLORINATED DI-BENZO-P-DIOXINS	
A Water: GC/MS	CE 1
B Fish: GC/MS	CE 14
CHLOROPHYLL	
A Spectrophotometry	CF 1
CHROMIUM	
A Hexavalent: Diphenylcarbazide - Spectrophotometric	CG 1
See also Trace Metals	
COBALT	
See Trace Metals	
COLOR	
A Apparent: Spectrophotometric	CH 1
B Visual Comparison	CH 9
CONDUCTIVITY	
A Radiometer Conductivity Meter	CI 1
B Electronic Switchgear Conductivity Meter	CI 9
COPPER	
See Trace Metals	
CYANIDE	
A Manual Distillation - Automated Colorimetry	CJ 1
B Automated Gas Dialysis - Colorimetry	CJ 11
DUSTFALL	
A Settleable Particulate - ASTM	DA 1
FLUORIDE	
A Automated Alizarin Blue Colorimetry	FA 1
B Specific Ion Electrode	FA 9
C Alkali Fusion - Automated Alizarin Blue Colorimetry	FA 14
D Fluoridation Rate of the Atmosphere: Candle - Specific Ion Electrode	FA 21
GOLD	
See Trace Metals	

TABLE OF CONTENTS

	Page
HARDNESS	
A Manual Titration	HA 1
B Semi Automated Titration	HA 9
See also Calcium, Magnesium, Trace Metals	
IRON	
A Autoclaved - Automated TPTZ Colorimetry	IA 1
See also Trace Metals, Manganese	
LEAD	
See Trace Metals	
LITHIUM	
See Trace Metals	
MAGNESIUM	
A Calculation	MA 1
B Automated Atomic Absorption	MA 4
See also Trace Metals	
MANGANESE	
A Automated Colorimetry	MB 1
See also Trace Metals, Iron	
MERCURY	
A Acid Digestion - Manual Flameless Atomic Absorption	MC 1
B Acid Digestion - Automated Flameless Atomic Absorption	MC 11
C Methyl Mercury: Gas Chromatography	MC 21
METALS - TRACE METALS	
Introduction	MD 1
1 Flame Atomic Absorption	MD 21
2 Flameless Atomic Absorption	MD 47
3 Inductively Coupled Plasma Atomic Emission	MD 54
4-1 D.C. Arc Atomic Emission - Qualitative	MD 66
4-2 D.C. Arc Atomic Emission - Semi-Quantitative	MD 75
5-1 X-ray Fluorescence Spectrometry - Lead in Air Particulate	MD 80
5-2 X-ray Fluorescence Spectrometry - Calcium on CoH Tapes	MD 86
5-3 X-ray Fluorescence Spectrometry - Ca, K, Cl, S, P, Si in Vegetation	MD 92
MOISTURE CONTENT	
A Indirect Gravimetric	ME 1
See also Organochlorine Pesticides and Polychlorinated Biphenyls	
MOLYBDENUM	
See Trace Metals	
NICKEL	
See Trace Metals	

TABLE OF CONTENTS

	Page
NITROGEN-AMMONIA	
A Automated Phenate-Hypochlorite-Colorimetry	NA 1
A-1 Variation # 1	NA 9
A-2 Variation # 2	NA 13
NITROGEN-NITRATE	
A Automated Ion Chromatography	NB 1
See also Nitrate plus Nitrite, Nitrite	
NITROGEN-NITRATE PLUS NITRITE	
A Hydrazine Reduction - Azo Dye Colorimetry	NC 1
A-1 Variation # 1	NC 11
NITROGEN-NITRITE	
A Azo Dye Colorimetry	ND 1
A-1 Variation # 1	ND 8
NITROGEN-TOTAL KJELDAHL	
A Block Digestion - Automated Neutralization and Colorimetry	NE 1
A-1 Variation # 1	NE 11
ORGANIC SOLVENT EXTRACTABLE MATTER	
A Liquid-Liquid Extraction	OA 1
B Liquid-Solid Extraction (Soxhlet)	OA 8
ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS	
A Gas Chromatography	OB 1
B Rapid Scan - Gas Chromatography	OB 24
C Routine Scan - Gas Chromatography	OB 30
D Silica Gel - Gas Chromatography	OB 34
ORGANO PHOSPHORUS PESTICIDES	
A Gas Chromatography	OC 1
OXYGEN DEMAND - BIOCHEMICAL (BOD)	
A Incubation - Oxygen Electrode	OD 1
OXYGEN DEMAND - CHEMICAL (COD)	
A Dichromate Method	OE 1
OXYGEN - DISSOLVED	
A Winkler Titration	OF 1
B Oxygen Electrode	OF 9
PARTICLE SIZE DISTRIBUTION	
A Sieve Analysis	PA 1
B Hydrometer Analysis	PA 11
C Pipet Analysis	PA 19

TABLE OF CONTENTS

	Page
PETROLEUM HYDROCARBONS	
A Gas Chromatography	PB 1
pH	
A Electrometric Method	PC 1
PHENOLIC COMPOUNDS	
A Automated 4-Aminoantipyrine Colorimetry	PD 1
PHENOXY ACID TYPE HERBICIDES	
A Gas Chromatography	PE 1
PHOSPHORUS	
A Filtered: Automated Ascorbic Acid Colorimetry	PF 1
A-1 Variation # 1	PF 8
B Autoclave - Automated Ascorbic Acid Colorimetry	PF 14
C Total: Acid Digestion - Automated Ascorbic Acid Colorimetry	PF 22
C-1 Total: Variation # 1	PF 30
D Liquid Scintillation Counting	PF 38
E X-ray Fluorescence	PF 42
F Total: Acid Digestion - Automated Molybdenum Blue Colorimetry	PF 43
See also Trace Metals (5-3)	
POLYNUCLEAR AROMATIC HYDROCARBONS	
A HPLC - Fluorimetry	PG 1
POTASSIUM	
A Automated Atomic Absorption	PH 1
See also Trace Metals (5-3)	
RESIDUE	
See Solids	
SELENIUM	
A Hydride Generation - Flameless Atomic Absorption	SA 1
SILICATES - MOLYBDATE REACTIVE	
A Automated Molybdate Colorimetry	SB 1
See also Trace Metals (5-3)	
SILVER	
See Trace Metals	
SODIUM	
A Automated Atomic Absorption	SC 1
See also Trace Metals	
SOLIDS - DISSOLVED	
A Gravimetric Method	SD 1
B Calculation from Conductivity	SD 7
C Summation of Ionic Species	SD 9

TABLE OF CONTENTS

	Page
SOLIDS - IGNITED	
A Gravimetric Method	SE 1
SOLIDS - SUSPENDED	
A Gravimetric Method	SF 1
SOLIDS - TOTAL	
A Calculation Method	SG 1
B Gravimetric Method	SG 3
STRONTIUM	
See Trace Metals	
SULPHATE	
A Methylthymol Blue - Automated Colorimetry	SH 1
B Automated Ion Chromatography	SH 13
SULPHIDE	
A Inert Gas Displacement - Ammonium Molybdate Colorimetry	SI 1
B Semi-Quantitative Hach Lead Acetate Test	SI 9
C Specific Ion Electrode	SI 13
D Methylene Blue Spectrophotometry	SI 18
SUSPENDED AIR PARTICULATE	
A High Volume Gravimetric Method	SJ 1
THALLIUM	
See Trace Metals	
TIN	
See Trace Metals	
TITANIUM	
See Trace Metals	
TOLUENE-2,4-DIISOCYANATE	
A Gas Chromatography	TA 1
TRACE METALS	
Introduction	MD 1
1 Flame Atomic Absorption	MD 21
2 Flameless Atomic Absorption	MD 47
3 Inductively Coupled Plasma Atomic Emission	MD 54
4-1 D.C. Arc Atomic Emission - Qualitative	MD 66
4-2 D.C. Arc Atomic Emission - Semi-Quantitative	MD 75
5-1 X-ray Fluorescence Spectrometry - Lead in Air Particulate	MD 80
5-2 X-ray Fluorescence Spectrometry - Calcium on CoH Tapes	MD 86
5-3 X-ray Fluorescence Spectrometry - Ca, K, Cl, S, P, Si in Vegetation	MD 92

TABLE OF CONTENTS

Page

TRIAZINE HERBICIDES

A	Water: Gas Chromatography	TB 1
B	Soils and Sediments: Gas Chromatography	TB 8

TURBIDITY

A	Nephelometric Method	TC 1
---	----------------------	------

VANADIUM

See Trace Metals

VINYL CHLORIDE

A	Gas Chromatography	VA 1
---	--------------------	------

VOLATILE ACIDS

A	Column Partition Chromatography	VB 1
---	---------------------------------	------

ZINC

See Trace Metals

MICROBIOLOGICAL PARAMETERS

(inserted at end of manual)

35°C HETEROTROPHIC BACTERIA

A	Membrane Filter Analysis	ML 1
---	--------------------------	------

POLLUTION INDICATOR BACTERIA

A	Presence Absence Test	MP 1
---	-----------------------	------

PSEUDOMONAS AERUGINOSA

A	Membrane Filter Analysis	MT 1
---	--------------------------	------

TOTAL COLIFORMS, FECAL COLIFORMS AND FECAL STREPTOCOCCI

A	Membrane Filter Analysis	MX 1
---	--------------------------	------

INTRODUCTION

This is the first edition of "Handbook of Analytical Methods for Environmental Samples". In it are contained methods in use by the Ministry of the Environment laboratories for the analysis of various parameters in a wide range of environmental samples. The different sample types for which a particular method is valid are listed in the method summary. Before any method is implemented, it is carefully tested and compared with other methods in common use.

Preservation techniques and the required sample containers are described briefly in each method. For more information on these facets of analysis and sampling techniques, the reader is referred to the following Ontario Ministry of the Environment publications:

"Outlines of Analytical Methods" (1981)

A guide to the occurrence, significance, sampling and analysis of chemical and microbiological parameters in water, sediment, soil, vegetation and air.

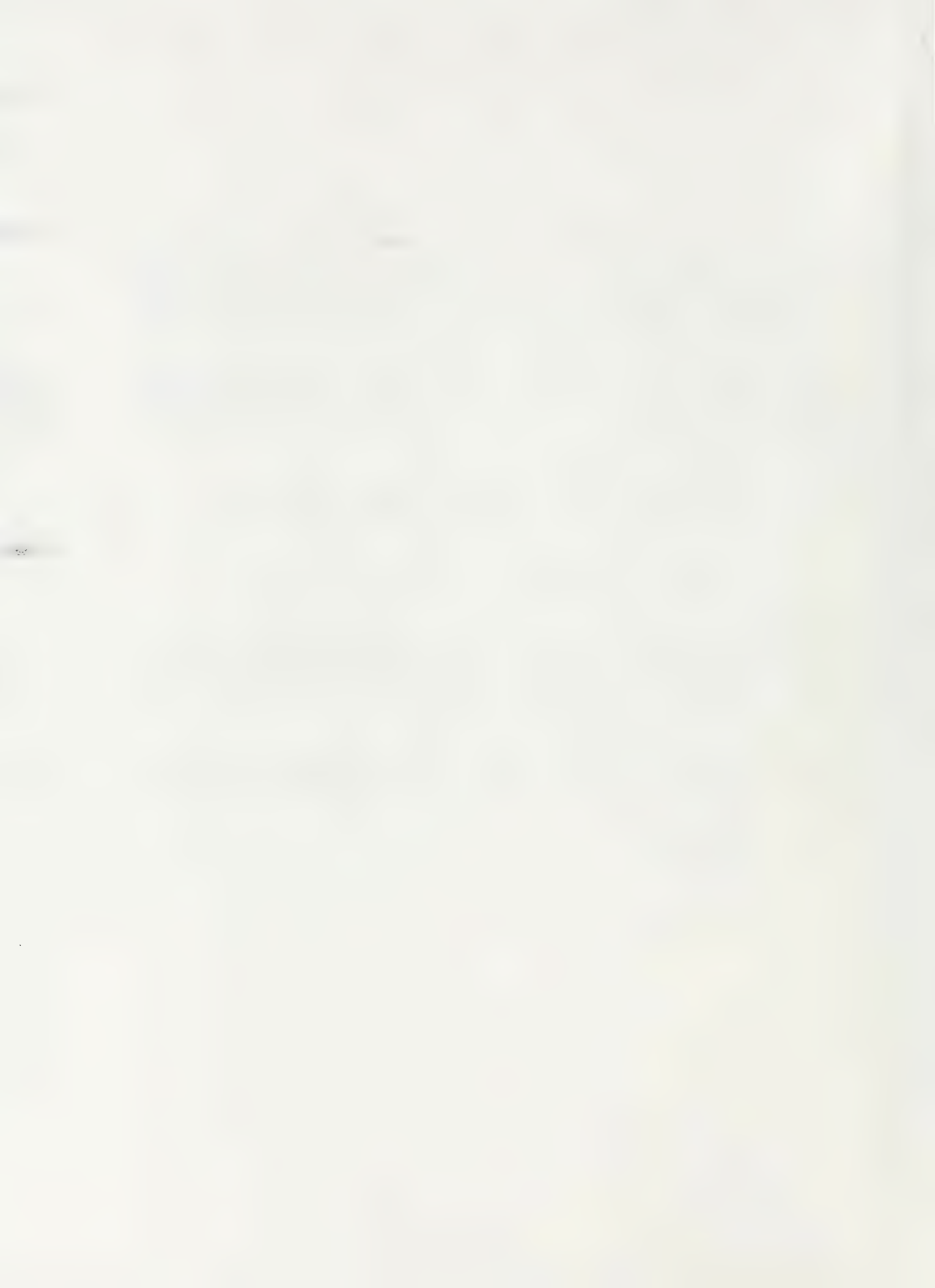
"Guide to the Collection and Submission of Samples for Laboratory Analysis" (1984)

The methods in this manual are as current as possible considering the length of time required to publish a manual such as this and the rapidly changing techniques and theories of analysis. The laboratory plans to update this manual every two years. The reader is requested to return the Distribution Record in this manual so that new material can be forwarded when available.

The laboratory has tried to provide complete details for each method in use. Any reader who requires any further information or has any knowledge of errors in our methods is invited to contact the Assistant Director's office, Laboratory Services.

Laboratory Services and Applied Research Branch
Ontario Ministry of the Environment
P.O. Box 213
Rexdale, Ontario
M9W 5L1

Telephone (416) 248-3512



QUALITY CONTROL

The major objective of a quality control program for chemical analysis is to ensure immediate detection of any deterioration in instrumentation, chemical reagents and standards, techniques and calibration.

Calibration is achieved by means of standardized solutions covering the range of instrument response and is performed before the analytical run commences. Since high degrees of both accuracy and precision are required to detect and minimize any between-run changes, standards are analyzed with as little handling or preparation as possible.

The calibration is confirmed by means of two control standards, QC-A and QC-B, which are made up and maintained independently of the calibration standards. The system is not calibrated with these control standards. In order to monitor both blank and slope biases, QC-A and QC-B are chosen to be about 70% and 10% of full scale respectively. Analyzing two QC standards in the upper range of the test monitors slope bias only. Analyzing both QC standards in the lower range will monitor blank bias only.

When the QC-A and QC-B control standards are analyzed and read, their sum and difference are calculated, plotted versus time on a control chart and used immediately by the technician to determine whether the calibration process is in control. This involves the concepts of Warning, Control and Acceptance Limits as defined by variation in the difference (D) between QC-A and QC-B standards. This is estimated by the standard deviation of the differences (S_D) from previous data and is a measure of within-run variation.

Warning Limit: If the current value of QC-A - QC-B differs from the defined value by more than $2 S_D$, then recent past values should be reviewed to determine if a trend towards out-of-control is developing. Analysis may proceed if this is not apparent. In general, the warning limits are not expected to be exceeded more than once in twenty times.

If the current value of QC-A + QC-B differs from the defined value by more than $2 S_D$, then systematic calibration error may be present. The recent trend in the plot of QC-A + QC-B should be examined carefully.

Control Limit: If the current value of QC-A - QC-B differs from the defined value by more than $3 S_D$, then today's calibration slope is out of control. Since these limits are not expected to be exceeded more than 1 time in 200, there is probably something wrong with the system. The analytical run must be stopped until a cause is determined. The system must be recalibrated and rechecked using QC-A and QC-B. On the short term, if the slope is in control, control limits for QC-A + QC-B should be set at $3 S_D$ to prevent significant "blank correction" bias where this is important.

Acceptance Limit: Systematic error in estimating slope and blank corrections will cause the sum (T) of QC-A and QC-B to vary excessively. Over the long term, in order to control between-run standard deviation, the standard deviation (S_T) of QC-A + QC-B should not exceed S_D by a factor of more than 1.5. Therefore if QC-A + QC-B exceeds the Acceptance Limit of $4.5 S_D$ the presence of excessive systematic error is suspected. The analytical run is stopped and the system is recalibrated.

The actual values of QC-A and QC-B are examined to see if either is suspect whenever their difference or sum is found to be out of control, but they are not themselves separately controlled. However, over the long term the standard deviations of QC-A and QC-B (S_A and S_B respectively) can be used to estimate the between-run standard deviation and its dependence on concentration. In many systems the in-run standard deviation is not particularly concentration dependent, although the between-run standard deviation may be. While the QC-A and QC-B process is useful for controlling this effect it is often easier said than done. The concept of the Acceptance Limit recognizes that data users do not always require the ultimate performance implied by the variation of in-run duplicates, and that there is much to be gained in terms of method evaluation by allowing calibration to drift in a controlled fashion.

In practice, the Acceptance Limit for Sums and the Control and Warning Limits for Differences may be tightened to achieve a particular benefit, but, in general, they should not be set too much tighter than the system can tolerate, and should never be set looser than long-term performance data suggests can be maintained routinely.

In methods where sample preparation such as digestion or extraction is required, a recovery check suitable to that system is required to estimate the efficiency of the analysis. These solutions are not used to calibrate the instrument, but corrections for the digestion blank and matrix effects can be estimated and applied if necessary. Recovery standards are chosen to test all facets of the analysis. If a digestion step is supposed to liberate a substance or convert one substance into another before analysis, the recovery standard should be chosen to test the efficiency of this step. For example, glycine is used to test the efficiency of the digestion in the total Kjeldahl nitrogen test. Recovery efficiency is generally reported as percent recovery.

Standard Methods states that precision refers to the reproducibility of a method when it is repeated on a homogeneous sample under controlled conditions, regardless of whether or not the observed values are widely displaced from the true value as a result of systematic or constant errors present throughout the measurements. Precision can be expressed by the standard deviation.

If precision is estimated from repeated analyses of a single standard or sample the standard deviation and the mean value measured are quoted. If precision is estimated from duplicate analyses of many samples, the standard deviation and concentration range employed for the statistical analysis are quoted. Generally, duplicates are broken down into 3 ranges which are expressed as percentages of full scale: 0-20%, 20-50% and 50-100%.

The Detection Criterion refers to the minimum analytical result which must be observed before it can be stated that a substance has been discerned with an acceptable probability that the statement is true. The risk of making a Type 1 error is 5%. The Detection Criterion is calculated by multiplying the standard deviation of an analytical procedure that has been determined at low concentrations including zero by 1.645. The Detection Limit is twice the Detection Criterion.

Accuracy refers to the agreement between the average of several measured values and the accepted or "true" value. Accuracy is generally expressed as the difference between these two values or as relative error where the difference is expressed as a percentage of the accepted value. Accuracy is estimated by analyzing standard reference materials. To test a laboratory's ability to analyze real samples, that laboratory should participate in round-robin intercomparisons.

All the procedures outlined so far deal primarily with the analysis of a single parameter. Real samples contain several parameters, many of which are interrelated. When all the parameters requested on a particular sample have been analyzed, the results are studied by a senior technician and may be subjected to ion balance and conductivity calculations if there are enough data. Where possible, samples can be checked for upstream/downstream consistency or against historical data.

Each member of the laboratory staff has a responsibility in the Quality Control/Quality Assurance program ranging from sample preparation, to instrument calibration, to result checking, to long term QC monitoring.

References:

1. Laboratory Services Branch, Data Quality Report Series, Section 1-A, The Water Quality Laboratories Data Quality Summary, 1975. P. Fellin and D.E. King.
2. Laboratory Services Branch, Data Quality Report Series, Section 1-B, The Water Quality Laboratories Performance Reports, 1975. P. Fellin and D.E. King.
3. Laboratory Services Branch, Detection of Systematic Error in Routine Trace Analysis, 1974. D.E. King.
4. Laboratory Services Branch, Data Quality Report Series, Quality Control Procedures and Objectives, 1976.
5. Laboratory Services Branch, Quality Control Report - 1980, Water Quality Section. W.B. Moody.
6. Laboratory Services Branch, Ionic Balance, Conductivity, Dissolved Solids and Langelier's Index Calculations from Analytical Data, 1982. M.W. Rawlings, Internal Report.
7. Laboratory Services Branch, Protocol for Quality Control in the Water Quality Section, 1983. W.B. Moody.
8. Clark, J.L., extract from "The Quality Control Handbook for Pilot Watershed Studies", PLUARG, IJC; revised March 13, 1980.
9. American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1980. Standard Methods for the Examination of Water and Wastewater. 15th Ed.



THE DETERMINATION OF ACIDITY

The acidity of water is a measure of its capacity to neutralize a base or its capacity to donate protons. Acidity is defined as the amount of base (expressed as mg/l calcium carbonate) required to raise the pH of the sample to 8.3, i.e. the "phenolphthalein endpoint". This corresponds to the point of carbonic acid - carbonate equivalence. At this pH the predominant inorganic carbon species is the bicarbonate ion, HCO_3^- .

The major component of acidity in natural waters is carbonic acid formed by the absorption of carbon dioxide. Other lesser components include: portions of other weak acids such as phenolic acid and undissociated tannic acid; hydrolyzable salts, particularly of iron and aluminum; and mineral acids causing an unusually low pH. Acidity is a capacity function and can only be interpreted in terms of specific substances when the chemical composition of the sample is known.

Precipitation is naturally slightly acidic since the reaction of atmospheric carbon dioxide with moisture forms carbonic acid, or more precisely, hydrated carbon dioxide. Frequently, rain and snow are further acidified when oxides of sulphur and nitrogen react chemically with atmospheric oxygen and moisture to form strong acids. In some cases, the strong acid fraction may be the major contributor to the total acidity of the precipitation sample.

Carbonic acid acidity is present in any water with a pH between 4.5 and 8.3. Below pH 4.5, mineral acidity predominates. The acidity of the natural water system is increased by industrial activity. For example, oxides of sulphur and nitrogen emitted to the atmosphere from industrial sources contribute to acid precipitation, and the bacterial degradation of pyrite which results in sulphuric acid and hydrolyzable iron renders acidic the drainage waters from mine tailings. Acid waters inhibit biological activity and have objectionable corrosive properties. At present, no acidity criterion exists for drinking water.

Sample Handling and Preservation

Water

Ideally sample bottles should be completely filled so that no air bubbles remain after capping and then stored at a low temperature. Bottles should not be allowed to freeze during transport because of the danger of breakage. Since samples may be subject to microbial action and to the loss or gain of carbon dioxide or other gases when exposed to air, the samples must be analyzed as soon as possible after collection.

Selection of Method

Drinking water, surface water and industrial waste samples are analyzed titrimetrically with a standard carbon dioxide free sodium hydroxide solution to a fixed endpoint of pH 8.3 (Method A). This endpoint determination can be made by using either a pH meter or phenolphthalein indicator. Precipitation samples are normally titrated automatically to

the same endpoint using a microprocessor controlled titration system comprised of a microcomputer, digital pH meter and an autoburette (Method B). Since this method produces a complete set of titration data for each sample, it is also possible to calculate the exact potentiometric endpoint of the titration by either of two mathematically independent methods. Normally, a Gran analysis is performed on the titration data producing a total inflection acidity result for each precipitation sample. As the component of strong acids relative to the carbonic acid concentration increases, this "inflection" acidity may be significantly different from the pH 8.3 endpoint acidity. The pH of the endpoint for strong acids is 7.0, therefore a fixed endpoint titration to pH 8.3 may overestimate the acidity of a sample.

ACIDITY

Manual Fixed Endpoint (FE) Titration Method A

SUMMARY

Matrix.	This method is used routinely on drinking and surface water and industrial waste samples.
Substance determined.	Acidity (total proton donation capacity) = (H^+ contributed by carbonic acid dissociation + hydrolysis of metal ions such as iron, aluminum and manganese + dissociation of strong acids such as sulphuric and nitric.)
Interpretation of results.	Although the results are reported as mg/l calcium carbonate, neither calcium nor carbonate are actually measured during the titration.
Principle of method.	Acidity of a water is a measure of its capacity to neutralize a strong base to a designated pH of 8.3. Strong mineral acids, weak acids such as carbonic acid and hydrolyzing salts may contribute to acidity. Total sample acidity is titrated against standard (carbon dioxide free) sodium hydroxide solution to a phenolphthalein or pH endpoint (pH 8.3).
Time required for analysis.	Approximately 60 acidity determinations may be completed per day.
Range of application.	0.1 - 50 mg/l acidity as calcium carbonate on undiluted samples assuming 100 ml is used; samples in excess of 50 mg/l calcium carbonate may be determined after appropriate dilution.
Standard deviation.	0.15 mg/l in the 0 - 10 mg/l as calcium carbonate range.
Accuracy.	Bias is controlled to within 3% throughout the working range.
Detection criteria.	0.25 mg/l acidity as calcium carbonate; by definition if the sample pH is greater than 8.3, it has no acidity.
Interferences and shortcomings.	Any substance that can be titrated by a base is measured by the test procedure. Low results may occur when too much indicator is added when using the phenolphthalein endpoint. Highly colored samples interfere with the ability to distinguish the endpoint.
Minimum volume of sample.	100 ml; sample volumes in excess of 100 ml are advised for rinsing of glassware.

**Preservation and
sample container.**

Either glass or plastic sample containers are satisfactory. Ideally the bottle should be filled completely so that no bubbles remain after capping and then stored at a low temperature. No preservative is recommended and freezing should be avoided.

**Safety
considerations.**

Normal care should be exercised when using the corrosive sodium hydroxide titrant (0.0200N, pH greater than 12). Eye protection should be worn in making up stock and working solutions.

ACIDITY

Manual Fixed Endpoint (FE) Titration Method A

1. Introduction

Hydrogen ions present in a sample as a result of dissociation or hydrolysis of solutes are neutralized by titration with standard alkali (0.0200N sodium hydroxide). The acidity thus depends on the endpoint pH or indicator used. Routinely, titrations are performed to a phenolphthalein or pH endpoint (pH 8.3). Acidity is expressed as mg/l calcium carbonate.

2. Interferences and Shortcomings

No significant interferences exist for the acidity determination although a fading phenolphthalein endpoint may be observed in highly colored samples. A pH endpoint detection system should be used whenever this occurs. Sufficient time for reaction of the titrant with slow hydrolyzable salts (iron and aluminum sulphate) should be allowed between dropwise additions of the sodium hydroxide near the endpoint. Protection of the titrant from absorption of atmospheric carbon dioxide by keeping reagent bottles stoppered and using only fresh sodium hydroxide in the burette is important.

3. Apparatus

- 3.1. Microburette, 5 ml, with reservoir.
- 3.2. pH meter with expanded scale.
- 3.3. pH electrodes.
- 3.4. Magnetic stirrer with stirring bars.
- 3.5. Berzelius beakers, 200 ml, (12).
- 3.6. Reagent bottles with ground glass stoppers.
- 3.7. Assorted volumetric pipettes.

4. Reagents

- 4.1. Sodium hydroxide (NaOH), reagent grade.
- 4.2. Potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$), primary standard grade.
- 4.3. Phenolphthalein indicator.

4.4. Stock Sodium Hydroxide Solution (1N)

Dissolve 40 g of sodium hydroxide pellets in 1 liter of boiled and cooled distilled carbon dioxide free water.

4.5. Working Sodium Hydroxide Titrant (0.02N)

Dilute 20 ml of stock sodium hydroxide solution to 1 liter with boiled and cooled distilled carbon dioxide free water. Standardize daily.

4.6. Standard Potassium Hydrogen Phthalate Reagent (0.0200N)

Dissolve 4.085 g anhydrous potassium hydrogen phthalate (dried at 120°C for 2 hours) in exactly 1 liter of carbon dioxide free distilled water.

4.7. Phenolphthalein Indicator Solution

Dissolve 0.5 g phenolphthalein in 50 ml 95% ethanol or isopropanol and dilute to 100 ml with distilled water.

Standardization

Pipette 5.0 ml of standard potassium hydrogen phthalate solution (0.0200N) into a 200 ml beaker. Bring volume up to 150 ml with distilled water. With continuous stirring, titrate the standard with working sodium hydroxide titrant to a pH endpoint of 8.3, or if using the phenolphthalein indicator, to a pink color which persists for 30 seconds.

5. Procedure

5.1. Pipette an appropriate volume of sample (normally 100 ml) into a 200 ml beaker containing a magnetic stirring bar.

5.2. pH Endpoint (Recommended Technique)

Titrate the sample, with continuous stirring, to a pH endpoint of 8.3 with standardized sodium hydroxide titrant.

OR

5.3. Phenolphthalein Endpoint

Add three drops of phenolphthalein indicator and with continuous stirring, titrate the sample with working sodium hydroxide titrant to a definite pink color which persists for 30 seconds.

6. Calculation and Reporting

Results are reported in mg/l as calcium carbonate (CaCO_3)

$$\text{Acidity} = \frac{V \times N \times 50,000}{V_s} \text{ mg/l CaCO}_3$$

Where:

V = ml of sodium hydroxide titrant

V_s = ml of sample used

N = normality of sodium hydroxide titrant as determined by duplicate (minimum) standardization against 0.0200N potassium hydrogen phthalate solution. Results are reported as follows:

Range	Report to
0.02 - 9.99 mg/l	0.01 mg/l increments
greater than 10.0 mg/l	3 significant figures

7. Precision and Accuracy

A standard deviation of 0.15 mg/l acidity as calcium carbonate can be expected for samples analyzed in duplicate in the 0 - 10 mg/l acidity as calcium carbonate range. Bias is controlled by analyzing 2 independantly prepared long-term standards and control is maintained within 3% throughout the working range.

8. Bibliography

- 8.1. Ministry of the Environment (1975). Outlines of Analytical Methods. Laboratory Services Branch, Rexdale, Ontario.
- 8.2. American Public Health Association, American Waterworks Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th ed. APHA, Washington, D.C. 273-277.

ACIDITY

Automated Titration Method B

SUMMARY

Matrix.	This method is used routinely for acidity determinations on precipitation samples.
Substance determined.	Acidity (total proton donation capacity) = (H^+ contributed by carbonic acid dissociation + hydrolysis of metal ions such as iron, aluminum and manganese + dissociation of strong acids such as sulphuric and nitric acids).
Interpretation of results.	Results are reported as mg/l calcium carbonate, although neither calcium nor carbonate are actually measured.
Principle of method.	Total sample acidity is titrated automatically against standard sodium hydroxide solution to a pH endpoint (pH 8.3). The potentiometric endpoint is also determined using the titration data.
Time required for analysis.	Approximately 15 minutes is required for a single analysis although this may vary with the acidity of the sample (samples having high acidity will require a longer time.) Approximately 30 acidity determinations may be completed per day.
Range of application.	It is recommended that samples in excess of 50 mg/l calcium carbonate be appropriately diluted prior to titration. The general range of precipitation samples has been found to be 0 - 20 mg/l acidity as calcium carbonate.
Standard deviation.	0.07 mg/l in the 0 - 10 mg/l acidity as calcium carbonate range.
Accuracy.	25.02 ± 0.27 for a standard containing 25.0 mg/l acidity as calcium carbonate.
Detection criteria.	0.10 mg/l acidity as calcium carbonate. By definition if the sample pH is greater than 8.3, it has no acidity.
Interferences and shortcomings.	The rate of titration and pH stability determine the precision and accuracy of the test. The presence of particulate matter may result in a standard deviation greater than that indicated above.
Minimum volume of sample	100 ml; however, sample volumes in excess of 100 ml are recommended for rinsing glassware. By special request it is possible for the laboratory to perform a titration on a 50 ml aliquot of sample.

**Preservation and
sample container.**

Pyrex glass or plastic sample containers are satisfactory. Ideally the bottle should be filled completely so that no bubbles remain after capping and then stored at a low temperature. No preservative is recommended and freezing should be avoided.

**Safety
considerations.**

Normal care should be exercised when using the corrosive sodium hydroxide titrant. Eye protection should be worn in making up stock and working sodium hydroxide solutions.

ACIDITY

Automated Titration Method B

1. Introduction

The acidity of a sample is determined by automatic titration of a sample aliquot with standard sodium hydroxide ($\approx 0.01N$) to a pH greater than 8.75. Gran, Differential and Total Fixed Endpoint (corresponding to pH of 8.3) acidities are calculated from the titration data. Titrant normality is determined by titration against standard 0.005N potassium hydrogen phthalate. The titrant delivery rate is controlled by the first derivative of the titration curve and by the stability of pH readings following each aliquot of titrant.

2. Interferences and Shortcomings

No significant interferences exist for the acidity determination although it is advisable to keep the sample vessel covered during the titration to minimize the effect of carbon dioxide exchange at the sample surface.

3. Apparatus

- 3.1. TRS-80 microcomputer complete with keyboard, video display, expansion interface, line printer and cassette recorder, or a PET microcomputer complete with line printer and disc drive.
- 3.2. Radiometer ABU 80 Autoburette with 2.5 ml total delivery burette assembly and BCD output.
- 3.3. Radiometer pHM 84 digital pH meter complete with BCD output.
- 3.4. In-house design interface/power supply box for interfacing the microcomputer to the Autoburette and pH meter.
- 3.5. pH electrodes. (Ingold low conductivity combination pH electrode recommended for precipitation samples.)
- 3.6. Magnetic stirrer with stirring bars.
- 3.7. Berzelius beakers, 100 ml and 200 ml capacity.
- 3.8. Reagent bottles (Pyrex glass with ground glass stoppers, or Nalgene with screw caps).
- 3.9. Assorted volumetric glassware (flasks and pipettes).

4. Reagents

4.1. Certified buffer solutions, pH 4.00, 6.86, 7.00 and 4.01.

4.2. Sodium hydroxide (NaOH), reagent grade pellets.

4.3. Potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$), primary standard grade.

4.4. Sulphuric acid (H_2SO_4) concentrated, reagent grade, or N/50 ampoules.

4.5. **Sodium Hydroxide Stock Solution (1N)**

Dissolve 40 g of sodium hydroxide pellets in 1 liter of carbon dioxide free distilled, deionized water (boiled and cooled).

4.6. **Working Sodium Hydroxide Titrant ($\approx 0.01\text{N}$)**

Dilute 10 ml of sodium hydroxide stock solution to 1 liter with carbon dioxide free distilled, deionized water.

4.7. **Standard Potassium Hydrogen Phthalate Reagent (0.005N)**

Dissolve 1.0212 g of anhydrous potassium hydrogen phthalate (previously dried at 120°C for 2 hours and cooled in a desiccator) in exactly 1 liter of carbon dioxide free distilled, deionized water (in a volumetric flask).

4.8. **Quality Control Buffer 6.86**

Dissolve commercially available salt according to package directions.

4.9. **Quality Control Buffer 4.01**

Dissolve 20.432 g of anhydrous potassium hydrogen phthalate (dried at 120°C for 2 hours and cooled in a desiccator) in 2 liters of distilled, deionized water.

4.10. **Quality Control Solution A - Stock (0.005N potassium hydrogen phthalate)**

Dissolve 1.0212 g of anhydrous potassium hydrogen phthalate in 1 liter of distilled, deionized water.

4.11. **Quality Control Solution A - Working QC-A (0.0005N potassium hydrogen phthalate)**

Dilute 100.0 ml of stock to 1 liter with distilled, deionized water. (Theoretical acidity = $500\text{ }\mu\text{eq/l}$ or 25 mg/l acidity as CaCO_3).

4.12. **Quality Control Solution B - Stock (0.02N H_2SO_4)**

Dilute 1 ampoule of N/50 concentrate to 1 liter according to package directions.

4.13. **Quality Control Solution B - Working QC-B (0.0002N H_2SO_4)**

Dilute 10.0 ml of stock to 1 liter with distilled, deionized water. (Theoretical acidity = $200\text{ }\mu\text{eq/l}$ or 10 mg/l acidity as CaCO_3).

5. Procedure

REFER TO THE MANUFACTURER'S MANUAL FOR GENERAL OPERATING PROCEDURES OF THE TRS-80 OR PET 2001 MICROCOMPUTER, ABU 80 AUTOBURETTE AND PHM 84 pH METER.

5.1. Preparation of the ABU 80 Autoburette

Fill the reservoir bottle of the ABU 80 autoburette with the 0.01N sodium hydroxide working titrant.

Protect the titrant from carbon dioxide absorption by using a carbon dioxide absorbent (e.g. Ascarite). Ensure that the Ascarite column is not blocked and that air may pass freely. If the reservoir bottle has been refilled or the titrant has been changed ensure that the burette delivery system is flushed with fresh titrant by pressing the FLUSH button on the autoburette. The normal operating conditions for the autoburette are:

- Power ON.
- Speed control set at 60.
- Ready light ON.
- MAN/AUTO control in the MAN position.
- Volume control in the 1/1 position.

5.2. Standardization of the PHM 84 pH Meter

REFER TO THE METHOD OF pH MEASUREMENT FOR A MORE DETAILED DISCUSSION.

- 5.2.1. Ensure that the electrodes are in good condition; that the reference filling solution is at the required level (replenish if necessary); and that the electrode filling hole is open to the atmosphere.
- 5.2.2. Pour an appropriate volume of pH 4.00 and 7.00 buffer into beakers, each containing a magnetic stirring bar.
- 5.2.3. Set the pH meter temperature dial to the appropriate temperature.
- 5.2.4. Set the ISO pH Control of the meter to 7.
- 5.2.5. Place the electrode in buffer 7.00 solution and while stirring, use the BUFFER CONTROL dial to adjust the pH meter reading to 7.000 ± 0.005 .
- 5.2.6. Remove the electrode from the buffer 7.00 solution and rinse with distilled water.
- 5.2.7. Place the electrode in buffer 4.00 solution and while stirring use the SLOPE CONTROL dial to adjust the pH meter reading to 4.000 ± 0.005 .
- 5.2.8. Check the adjustment several times by repeating steps 5.2.5 to 5.2.7 until no further adjustment is necessary and record the final SLOPE reading.

- 5.2.9. Read pH 6.86 and 4.01 buffers as Quality Control solutions and record the pH readings for each.

5.3. Preparation of the TRS-80 Microcomputer system

- 5.3.1. Turn on the microcomputer system in the following order:
- Interface/Power supply box (in-house design).
 - TRS-80 Keyboard.
 - TRS-80 Video Display.
 - TRS-80 Line Printer.
 - TRS-80 Expansion Interface.
- 5.3.2. When MEMORY? shows on the screen, press ENTER on the keyboard and READY> will appear. If the MEMORY? or READY> fails to appear hold the BREAK key and press the RESET button on the back of the keyboard.
- 5.3.3. Load the appropriate program using the CTR 80 recorder, or if available, using the TRS-80 disc drive. Refer to the User's Manual for details regarding loading procedure.
- 5.3.4. Enter RUN and the appropriate data via the TRS-80 Keyboard as requested. When START? appears, press ENTER to start the titration.

Preparation of the PET 2001 Microcomputer System

- 5.3.5. Turn on the microcomputer system in the following order:
- Interface/power supply box (in-house design)
 - PET Keyboard/Video
 - PET Line Printer
 - PET Disc Drive
- 5.3.6. Load the Appropriate program via the disc drive. Refer to the User's Manual for details regarding loading procedure.
- 5.3.7. Once the program is loaded type in RUN and press RETURN. Enter the appropriate data as requested on the screen, pressing RETURN after each entry.

5.3.8. Comments on use of both microcomputers

To interrupt execution at any time, use the BREAK key on TRS-80 or SHIFT and RUN/STOP keys on PET. To resume operation enter RUN then enter requested parameters.

If, on the PET, RETURN is hit without an input character, program execution is halted. To continue enter CONT.

There is no need to re-load the program on either system unless the power has been turned off.

A maximum of 150 data points are permitted for each titration.

The programs calculate inflection points during the titration. These appear on the display designated by an asterisk (*). These points are interpolated on the titration curve and, therefore, pH and volume

appear "out of order" with respect to the remainder of the points. The inflection points are not printed in the titration table.

5.4. Standardization of Working Titrant ($\approx 0.01N$ sodium hydroxide).

- 5.4.1. Pipette 10.0 ml of standard potassium hydrogen phthalate solution ($5.00 \times 10^{-3}N$) into a 200 ml beaker. Adjust the volume to 100 ml with distilled, deionized water. Titrate with working sodium hydroxide titrant using the microcomputer controlled system. Repeat the analysis for a duplicate sample of standard potassium hydrogen phthalate. Calculate the normality of the working sodium hydroxide titrant according to the procedure outlined in Section 6.
- 5.4.2. Interrupt the execution of the program after the standardization procedure and re-enter the appropriate data as requested on the Video Screen. The normality of base required in the program is that calculated for the working sodium hydroxide titrant from standardization against potassium hydrogen phthalate.

5.5. Titration of Samples

- 5.5.1. Pipette an appropriate volume of sample or Quality Control Standard (usually 100 ml) into a clean beaker containing a stirring bar and place on a stirrer. Set stirrer at a rate which does not create a vortex. An aliquot of each working QC-A and QC-B solution should be titrated daily and the data recorded.
- 5.5.2. Lower the electrode assembly into the sample ensuring that the electrode tip is clear of the stirring bar. In order to achieve pH stability it is necessary to adjust the relative heights of the delivery tip and pH electrode. Place the delivery tip as close as possible to the stirring bar and keep the electrode near the surface. Ensure that the porous plug of the electrode is below the surface of the sample.
- 5.5.3. After each titration is complete, press N for new sample or P for page feed and reply Y(yes) or N(no) for the same or new conditions when requested. Press BREAK on the TRS-80 or SHIFT and RUN/STOP on the PET when all samples are complete.
- 5.5.4. Power-off the equipment in the reverse order to the power-on sequence described in section 5.3.1. or 5.3.5.
- 5.5.5. Replace the rubber band over the filling hole of the electrode and store the electrode in the solution recommended by the manufacturer (distilled water for Ingold electrodes or pH 7 buffer for Radiometer electrodes).

6. Calculation and Reporting

The normality of the working sodium hydroxide titrant is determined by duplicate (minimum) standardization against 10 ml of $5.00 \times 10^{-3}N$ potassium hydrogen phthalate solution as follows:

$$N_{NaOH} = \frac{10.00 \times 0.005}{V}$$

Where:

v = Gran endpoint volume (in ml) given in the titration table printout (i.e. volume of sodium hydroxide titrant needed to raise the pH of the standard potassium hydrogen phthalate solution to its inflection point.

The FE acidity printed out in the titration table for each sample is reported to 3 significant figures.

The hard copy printout also provides the complete titration data set (pH vs cumulative volume of titrant added) and the inflection point acidity determined by both the GRAN and DIFFERENTIAL methods. In the case of precipitation samples this data provides the more accurate estimate of the sample's acidity.

Sample acidities are calculated as follows:

$$\text{Acidity} = \frac{V \times N_{\text{NaOH}}}{V_s} \text{ eq/l} = \frac{V \times N_{\text{NaOH}} \times 10^6}{V_s} \mu \text{ eq/l}$$

or to express acidity as mg/l CaCO_3 :

$$\text{Acidity} = \frac{V \times N_{\text{NaOH}}}{V_s} \times 50000 \text{ mg/l CaCO}_3$$

Where:

V = titrant volume (ml) (i.e. volume of titrant required to reach the specified endpoint)

N_{NaOH} = titrant normality

V_s = sample volume (ml)

7. Precision and Accuracy

The automated titration method exhibits a standard deviation of 0.07 mg/l acidity as calcium carbonate in the 0 - 10 mg/l acidity as calcium carbonate range. Some sample duplicates may deviate from this criterion due to the presence of particulate matter. For a standard containing 25.0 mg/l acidity as calcium carbonate, 25.02 ± 0.27 mg/l was obtained.

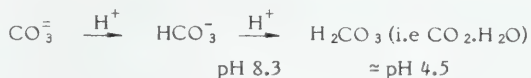
8. Bibliography

- 8.1. Ministry of the Environment (1975). Outline of Analytical Methods. Laboratory Services Branch, Rexdale, Ontario.
- 8.2. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th ed. APHA, Washington, D.C. 273-277.

THE DETERMINATION OF ALKALINITY

Alkalinity is defined as the capacity of a solution to neutralize acid. Alkalinity is a capacity function which has a different chemical basis than the concentration function, pH. A solution having a neutral pH may have considerable titratable alkalinity.

Any ion entering into a neutralization reaction with strong acid can contribute to alkalinity as long as the reaction takes place above the pH of the specified end point. Bicarbonate and carbonate are usually the major contributors to alkalinity of natural waters, due to the relative abundance of carbonate minerals and the availability of carbon dioxide which forms chemical equilibria with dissolved carbonates in water. Alkalinity values of such waters can be interpreted as being a result of concentrations of bicarbonate and carbonate ions alone, the titration reaction being:



Generally, this also means that the more alkalinity a body of water possesses, the greater its buffering capacity. Other anions imparting alkalinity to water include hydroxides, which, although rarely present in natural waters, may occur in water that has been softened by the lime or lime-soda process, or which has been in contact with cement. Also contributing to alkalinity are suspended matter; fluorides, silicates, phosphates, borates and organics present in some natural waters; and arsenate, aluminate and cyanide present in some industrial waste water. Alkalinity measures the hydrolyzable equivalent of all these substances.

Historically, a pH of 4.5 has been used for the alkalinity titration and is known as the total fixed end point (FE) alkalinity. A more precise alkalinity determination uses the inflection point of the titration curve. This is referred to as the total inflection end point (IP) alkalinity. For high alkalinity (hard water) samples the FE technique yields reliable results (i.e. FE ≈ IP), but for low alkalinity samples the true alkalinity value is overestimated if the FE method is used. In fact, samples containing 15 mg/l calcium carbonate have an end point closer to pH 5.1 - 5.3. Water bodies having a IP alkalinity of less than 15 mg/l calcium carbonate have a potential for acidification.

Sample Handling and Preservation

Water, Sewage and Industrial Waste

Samples containing large amounts of carbon dioxide may be unstable due to the possible loss of carbon dioxide gas. The loss may be minimized by filling the sample container to the neck and keeping it tightly closed and refrigerated until the test is performed. (Freezing should be avoided to protect against container breakage.)

Extremely caustic samples must not be kept in glass containers as silicate may be leached from the glass surface causing a change in the alkalinity of the sample. Soft water samples with low alkalinities should not be kept in soft glass containers for fear of leaching. Pyrex glass bottles with ground glass stoppers or plastic containers are mandatory.

Selection of Method

Although a manual colorimetric procedure can be applied to samples which are free of residual chlorine, color and turbidity, the visual detection of the end point is often not very precise. An automated potentiometric titration method is therefore used for the determination of FE alkalinity on drinking and surface waters and sewage and industrial waste samples. The potentiometric method is precise and free from residual chlorine, color and turbidity interferences. For drinking water, sewage and industrial waste samples, a semi-automated method is used whereby an aliquot is pipetted manually and automatically titrated to the 4.5 pH end point using a Fisher Titralyzer. Many surface water samples are automatically pipetted and titrated to the 4.5 pH end point using a Radiometer ATS-1 titration system (Method A).

Precipitation samples and some soft water river and lake samples (those with suspected low alkalinities) are analyzed for total inflection end point (IP) alkalinity. A true measure of the buffering capacity is found by the mathematical analysis of a complete titration curve to determine the location of the inflection point. This is achieved by a computer controlled titration system comprised of a microcomputer, digital pH meter and an autoburette. A known volume of sample is titrated with standard acid while measuring the solution pH. Upon completion of the titration, the Gran method of data evaluation is used to determine the end point volume which is then used to calculate the IP alkalinity (Method B).

ALKALINITY

Semi-Automated and Automated Fixed End Point (FE) Potentiometric Titration Method A

SUMMARY

Matrix.	The semi-automated Fisher Titralyzer method is used routinely for alkalinity determinations on drinking water, sewage and industrial waste samples. Many surface waters are analyzed by the automated method using a Radiometer ATS-1 titration system.
Substance determined.	Bicarbonate and carbonate, the main alkaline components normally found in natural waters, are measured. Any substance which will react to neutralize a strong acid above a pH of 4.5, will also be included.
Interpretation of results.	Alkalinity is reported as calcium carbonate equivalent in mg/l, however this does not imply that calcium carbonate is present. In addition to bicarbonate and carbonate a small part of the alkalinity in natural waters may be due to some forms of silica, phosphorus or boron, organic acid salts or suspended matter. Alkalinity in industrial wastes and sludge may be due in large part to hydroxide and hydrolyzable salts of metals such as iron and aluminum. Caution must be exercised when interpreting results on such samples in terms of specific chemical components.
Principle of method.	An aliquot of sample is pipetted either automatically or manually and automatically titrated with standard 0.02N sulphuric acid to an end point of pH 4.5, the approximate end point at which carbonate and bicarbonate are completely converted to carbonic acid. Some samples may contain titratable hydroxide and carbonate alkalinity. For these samples a preliminary titration to 8.3 is required. The analysis of sludges requires a preliminary centrifugation and filtration step to remove suspended particulates.
Time required for analysis.	Approximately 3 minutes are required for a single analysis using the automated potentiometric titration method. On a routine basis about 150 tests can be performed in a day. The semi-automated potentiometric titration takes slightly longer requiring 4 minutes for a single analysis allowing 100 analyses a day.
Range of application.	Levels of between 1.1 and 400 mg/l alkalinity as calcium carbonate, based on 50 ml of sample and a 50 ml burette, can be measured by the semi-automated method. Concentrations of between 0.7 and 200 mg/l alkalinity as calcium carbonate, based on a 10 ml sample and a 2.5 ml burette, can be measured by automatic pipetting and titration.

Standard deviation.	Based on duplicate drinking water samples using the semi-automated Fisher Titralyzer method; typical within-run standard deviations are: 0.68 in the 1.1 to 80 mg/l range; 0.98 in the 80 - 200 mg/l range; 1.70 in the 200 - 400 mg/l range. Based on within-run duplicate samples using the automated Radiometer ATS-1 method typical standard deviations are: 0.439 in the 0.7 - 40 mg/l range; 0.552 in the 40 - 100 mg/l range; 0.586 in the 100 - 200 mg/l range.
Accuracy.	Recoveries of Quality Control standards were 100% and 105% respectively for the Fisher instrument and 100% and 102% respectively for the Radiometer ATS-1.
Detection criteria.	1.12 mg/l alkalinity as calcium carbonate by the semi-automated Fisher Titralyzer method; 0.72 mg/l alkalinity as calcium carbonate by the automated Radiometer ATS-1 method. By definition, a sample with a pH of less than 4.5 has no alkalinity.
Interferences and shortcomings.	The titration itself produces carbon dioxide which, in solution, acts as an acid and depresses the pH. This effect is reduced if very little alkalinity is present since little carbon dioxide is produced. Carbon dioxide degassing during the titration may also occur, resulting in the occurrence of the end point at a higher pH.
Minimum volume of sample.	75 ml for the semi-automated Fisher Titralyzer II system. 25 ml for automated Radiometer ATS-1 system.
Preservation and sample container.	Polyethylene bottles or pyrex glass bottles with ground glass stoppers are mandatory for low level alkalinities and aggressive waters. Silicate leaching may occur with soft glass containers. Bottles should be filled to capacity and tightly capped to prevent carbon dioxide loss, and should be refrigerated. Avoid freezing because of the danger of container breakage.
Safety considerations.	Standard laboratory precautions should be taken when handling sulphuric acid. Samples containing cyanide will liberate cyanide gas (HCN) when titrated. These samples should be titrated manually in a fumehood.

ALKALINITY

Semi-Automated and Automated Fixed End Point (FE) Potentiometric Titration Method A

1. Introduction

An aliquot of sample is titrated automatically with 0.02N sulphuric acid to a specified pH end point of 4.5. The alkalinity is calculated from the volume and normality of titrant used and is reported as mg/l calcium carbonate.

2. Interferences and Shortcomings

By definition, anything titratable to 4.5 is included in the alkalinity measurement. It should be remembered that many anions, other than bicarbonate and carbonate, can contribute to alkalinity.

A potential source of error in this test, is the possible loss of carbon dioxide during the titration. This loss is minimized by rapid titration. In addition, the titration itself produces carbon dioxide which may depress the pH. The presence of particulates in the sample may result in an increase in titration time and partial titration of the particles.

3. Apparatus

3.1. Fisher Titralyzer system with 50 ml burette (semi-automated method)

OR

3.2. Radiometer ATS-1 autopipetting titration system with 2.5 ml burette (automated method).

3.3. Indicating electrode, pH glass electrode adaptable to the instrument for acid-base titrations.

3.4. Reference electrode, calomel electrode containing a saturated solution of potassium chloride.

3.5. Berzelius beakers, to fit the Fisher Titralyzer, with 1/8 inch flattened rims (48).

3.6. pH meter, laboratory or research model.

3.7. (Sludge samples only) IEC HN-S centrifuge capable of attaining 2000 rpm and handling 100 ml aliquots; equipped with speed control, brake and timer.

4. Reagents

- 4.1. Sodium carbonate (Na_2CO_3), anhydrous, reagent grade.
- 4.2. Sulphuric acid (H_2SO_4), concentrated reagent grade.
- 4.3. Buffer solution, pH 4.00, commercial preparation.
- 4.4. Buffer solution, pH 7.00 or pH 6.86, commercial preparation.
- 4.5. Saturated potassium chloride (KCl) solution.
- 4.6. **Standard Sodium Carbonate Solution (0.0200N)**

In a one liter volumetric flask, dissolve 1.060 g anhydrous sodium carbonate previously oven dried at 140°C , in a small amount of distilled water. Fill to the mark and store the solution in a glass reagent bottle.

- 4.7. **Sulphuric Acid Stock Solution ($\approx 0.8\text{N}$)**

Dilute approximately 360 ml concentrated sulphuric acid to 16 liters with distilled water.

Caution: Wear eye and hand protection.

- 4.8. **Standard Sulphuric Acid Titrant (0.0200N)**

Dilute approximately 400 ml of the sulphuric acid stock solution to 16 liters with distilled water. Standardize against standard 0.0200N sodium carbonate solution.

Caution: Wear eye and hand protection.

Standardization

Standardize a pH meter with the pH 4.00 buffer and check the calibration with the pH 7.00 or the pH 6.86 reference buffer. Pipette 20 ml of the 0.0200N sulphuric acid titrant into a 200 ml beaker. Introduce the pH electrodes, stirring the solution continuously, and titrate the acid with standard 0.0200N sodium carbonate to the pH 4.5 end point. Record the titrant volume. Repeat this procedure twice more and calculate the average normality of the acid. Adjust the acid concentration, if necessary, to 0.0200N and repeat the standardization.

- 4.9. **Quality Control Solutions**

For the Fisher Titralyzer system, two quality control solutions are prepared using sodium carbonate so that the concentrations are 285 mg/l alkalinity as calcium carbonate (QC-A) and 57 mg/l alkalinity as calcium carbonate (QC-B).

For the Radiometer ATS-1 system a 100 mg/l calcium carbonate equivalent standard is prepared and used for standardization of the titrant. The QC-A used is a water which has a relatively constant calcium carbonate equivalent of about 90 mg/l alkalinity as calcium carbonate and the QC-B is a 1:1 ratio of this water to distilled water and has an alkalinity as calcium carbonate of 45 mg/l.

5. Procedure

REFER TO THE MANUFACTURER'S MANUAL FOR GENERAL OPERATING PROCEDURES OF THE TITRATION EQUIPMENT AND pH METER.

- 5.1. Clean the reservoir with distilled water, and dry.
- 5.2. Place the 0.0200N sulphuric acid titrant into the reservoir. Cycle the titrator several times to flush the delivery lines with fresh titrant.
- 5.3. Calibrate the titrator for pH measurement with a buffer solution of pH 4.00 and check the scale with a pH 7.00 or pH 6.86 buffer.
- 5.4. Set the end point potential to the specified pH: pH 4.5 for total alkalinity; pH 8.3 for phenolphthalein alkalinity.
- 5.5 If sludge samples are to be analyzed the following pretreatment must be used:
 - 5.5.1. Centrifuge approximately 75 ml of sludge for 10 minutes at full speed. Decant supernatant.
 - 5.5.2. Pipette an appropriate aliquot (5 - 10 ml) of supernatant into a 200 ml Berzelius beaker, and dilute to approximately 50 ml with distilled water. Analyze according to 5.6.

5.6. Semi-automated Titralyzer System

- 5.6.1. Accurately pipette appropriate volumes of samples and required control solutions into 200 ml Berzelius beakers. The number of beakers prepared for each run should correspond to the number of spaces in the turntable.

NOTE: For sample volumes less than 50 ml add distilled water to the 50 ml mark to ensure that the electrodes will be immersed in the solution.

- 5.6.2. Record the sample numbers and their respective volumes and place the beakers in the correct order in the turntable assembly.
- 5.6.3. Start the automatic operation by carefully lowering the electrode assembly into the first beaker.
- 5.6.4. When the set of samples has been titrated, advance the tape and put the next series of samples onto the turntable. Repeat steps 5.6.1. to 5.6.4.
- 5.6.5. From the printed tape, record the amount of titrant opposite each corresponding sample number on bench sheets.

5.7. Automated ATS-I System.

- 5.7.1. Fill clean sample cups with sample or control solutions. Ensure that the sample order is recorded. Maintain the order as each cup is placed in position in the ATS-I sample belt.
- 5.7.2. Start the automatic operation by transporting the first sample cup into the pipetting station, and pressing the START BUTTON.
- 5.7.3. From the printed tape, record the amount of titrant opposite the recorded sample number or control standard on bench sheets.

NOTE: If more than one burette of titrant is required to titrate a sample, dilute the sample and place it in a sample cup at the end of the run. Record the dilution factor with the sample number.

6. Calculation and Reporting

Phenolphthalein alkalinity as mg/l CaCO_3

$$= \frac{a \times N \times 50,000}{v}$$

FE alkalinity as mg/l CaCO_3

$$= \frac{b \times N \times 50,000}{v}$$

Where:

a = ml titrant to reach pH 8.3 (phenolphthalein end point).

b = ml titrant to reach pH 4.5 end point.

N = normality of titrant.

v = volume of sample in ml.

For a theoretical classification of three principal forms of alkalinity the following table should be used:

Table I - Alkalinity Relationships

Results	Hydroxide Alkalinity	Carbonate Alkalinity	Bicarbonate Alkalinity
P=0	0	0	Total
P<1/2 Total	0	2P	Total - 2P
P=1/2 Total	0	2P	0
P>1/2 Total	2P - Total	2 (Total - P)	0
P=Total	Total	0	0

Where: P = phenolphthalein alkalinity

The results are reported in the following manner:

Range	Report to
0 (pH \leq 4.5)	report alkalinity as 0
1 - 9 mg/l	1 significant figure
10 - 99 mg/l	2 significant figures
100 mg/l and greater	3 significant figures

7. Precision and Accuracy

For the semi-automated Titrlyzer system standard deviations based on within-run duplicate samples are: 0.68 for the 1.1 to 80 mg/l range; 0.98 for the 80 - 200 mg/l range; and 1.70 in the 200 - 400 mg/l range.

For the automated ATS-1 system standard deviations based on within-run duplicate samples are 0.439 in the 0.7 - 40 mg/l range; 0.552 in the 40 - 100 mg/l range; and 0.586 in the 100 - 200 mg/l range.

Recoveries of Quality Control standards were 100% and 105% respectively for the Fisher instrument and 100% and 102% respectively for the Radiometer ATS-1

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1980). Standard Methods for the Examination of Water and Wastewater. 15th ed. APHA, Washington, D.C. 253-257.
- 8.2. United States Geological Survey (1970). Study and interpretation of the chemical characteristics of water. U.S. Geological Survey, Water Supply Paper No. 1473, U.S. Dept. of the Interior. 363 p.

ALKALINITY

Automated Inflection Point (IP) Potentiometric Titration Method B

SUMMARY

Matrix.	This method is used routinely on precipitation samples and river and lake samples with suspected low alkalinities (<15 mg/l alkalinity as calcium carbonate).
Substance determined.	The test is designed to measure the true buffering capacity of natural waters. Although bicarbonates are the main components, any substance which will react to neutralize a strong acid above the pH of the final inflection point, will also be included.
Interpretation of results.	Alkalinity is a measure of a gross property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known. Although results are generally reported as mg/l calcium carbonate this does not imply that calcium carbonate is the only species present or that it is in fact present at all.
Principle of method.	An aliquot of sample is titrated automatically with standard 0.0200N sulphuric acid and a complete titration curve obtained. The Gran method of analysis is used on the titration data to obtain the true buffering capacity (IP alkalinity) of the sample.
Time required for analysis.	Approximately 15 minutes are required for a single analysis although this varies depending on the alkalinity of the sample. On a routine basis about 30 tests can be performed in a day.
Range of application.	0.01 mg/l - 25 mg/l alkalinity as calcium carbonate.
Standard deviation.	0.05 mg/l alkalinity as calcium carbonate in the 0 - 10 mg/l alkalinity as calcium carbonate range based on replicate analysis of a composite sample.
Accuracy.	Bias control limits have not yet been established.
Detection criteria.	0.10 mg/l alkalinity as calcium carbonate; by definition if the original sample pH is lower than the pH of the inflection point, the sample has a "negative" alkalinity.

Interferences and shortcomings.

No interferences exist. The rate of titration and pH stability determine the precision and accuracy of the test. The presence of particulate matter may result in a standard deviation greater than that indicated above and an increased titration time since the particles are partially titrated.

Minimum volume of sample.

100 ml; however, sample volumes in excess of 100 ml are recommended for rinsing of glassware.

Preservation and sample container.

Either Pyrex glass bottles with ground glass stoppers or plastic sample containers are satisfactory. Ideally the bottle should be filled to capacity so that no bubbles remain after capping, and then stored at a low temperature. No preservative is required and freezing should be avoided.

Safety considerations.

Routine laboratory safety precautions. Concentrated sulphuric acid must be handled with care to prevent severe burns. Safety glasses must be worn during its use.

ALKALINITY

Automated Inflection Point (IP) Potentiometric Titration Method B

1. Introduction

A microcomputer has been interfaced with an autoburette and a digital pH meter to provide an automated titration system and data evaluation for IP alkalinity. An aliquot of sample (usually 100 ml) is titrated with standard sulphuric acid (0.0200N) under the control of a microcomputer. The titrant delivery rate is controlled by the first derivative of the titration curve and by the stability of pH readings following each aliquot of titrant. This allows for the smallest additions of titrant to be added when the titration curve is the steepest (i.e., nearest the inflection point). Once a complete titration curve is obtained, the Gran method of analysis is used to determine the end point volume and this in turn, is used to calculate the IP alkalinity. The result is reported in mg/l alkalinity as calcium carbonate.

2. Interferences and Shortcomings

No interferences exist with the potentiometric titration method. It should be noted that many anions, other than bicarbonates can contribute to alkalinity. Particulate matter may be partially titrated and may slow the titration.

3. Apparatus

- 3.1. TRS-80 microcomputer complete with keyboard, video display, expansion interface, line printer and cassette recorder or a PET microcomputer complete with line printer and disc drive.
- 3.2. Radiometer ABU 13 or ABU 80 Autoburette with 2.5 ml total delivery burette assembly and BCD output.
- 3.3. Radiometer pHM 64 or pHM 84 digital pH meter complete with BCD output.
- 3.4. In-house design interface/power supply box for interfacing the microcomputer to the Autoburette and pH meter.
- 3.5. pH electrodes.
- 3.6. Magnetic stirrer with stirring bars.
- 3.7. Berzelius beakers, 200 ml capacity.
- 3.8. Reagent bottles, Pyrex glass with ground glass stoppers, or Nalgene with screw caps.
- 3.9. Assorted volumetric glassware (flasks and pipettes).

4. Reagents

- 4.1. Certified buffer solutions, pH 4.00, 7.00, 6.86 and 9.00.
- 4.2. Sulphuric acid (H_2SO_4), concentrated volumetric solution: for N/50 preparation (ampoule size).
- 4.3. **Standard Sulphuric Working Titrant (0.0200N)**
Carefully transfer the contents of 1 ampoule of the concentrated sulphuric acid volumetric solution to a 1 liter volumetric flask. Rinse the ampoule several times with distilled, deionized water and add the rinsings to the flask. Dilute to the mark with distilled, deionized water.
- 4.4. **Standard sodium carbonate solution (0.0200N)**
In a 1 liter volumetric flask, dissolve 1.060 g anhydrous sodium carbonate previously oven dried at 140°C in a small amount of distilled water. Dilute to the mark and store the solution in a glass reagent bottle.

Standardization

Standardize a pH meter with the pH 4.00 buffer and check the calibration with the pH 7.00 or the pH 6.86 reference buffer. Pipette 20 ml of the 0.0200N sulphuric acid titrant into a 200 ml beaker. Introduce the pH electrodes, stirring the solution continuously, and titrate the acid with standard 0.0200N sodium carbonate to the pH 4.5 end point. Record the titrant volume. Repeat this procedure twice more and calculate the average normality of the acid.

5. Procedure

REFER TO THE MANUFACTURER'S MANUAL FOR GENERAL OPERATING PROCEDURES OF THE MICROCOMPUTER, AUTOBURETTE AND pH METER.

5.1. Preparation of the Autoburette

Fill the reservoir bottle of the Autoburette with the 0.0200N sulphuric acid working titrant. If the reservoir bottle has been filled or the titrant has been changed ensure that the burette delivery system has been flushed by depressing both the "EMPTY" and "AUTOREFILL" buttons and allowing the Autoburette to cycle at least three times if an ABU 13 autoburette is being used. If an ABU 80 autoburette is used, depress the FLUSH button.

5.2. Standardization of the pHM 64 or pHM 84 pH Meter

REFER TO THE METHOD OF pH MEASUREMENT FOR A MORE DETAILED DISCUSSION.

- 5.2.1. Ensure that the electrodes are in good condition; that the reference filling solution is at the required level (replenish if necessary); and that the electrode filling hole is open to the atmosphere.
- 5.2.2. Pour an appropriate volume of pH 4.00 and 7.00 buffers into beakers, each containing a magnetic stirring bar.

- 5.2.3. Set the pH meter temperature dial to the appropriate temperature.
- 5.2.4. Set the ISO pH Control of the meter to 7.
- 5.2.5. Place the electrode in buffer 7.00 solution while stirring and adjust the pH meter reading to 7.000 ± 0.005 using the BUFFER control dial.
- 5.2.6. Remove the electrode from the buffer 7.00 solution and rinse with distilled water.
- 5.2.7. Place the electrode in buffer 4.00 solution while stirring, and adjust the pH meter reading to 4.000 ± 0.005 using the SLOPE control dial.
- 5.2.8. Check the adjustment several times until no further adjustment is necessary and record the final SLOPE reading.
- 5.2.9. Read the pH 9.00 and 6.86 buffers as Quality Control solutions A and B and record the pH readings for each.

5.3. Preparation of the TRS-80 Microcomputer System

- 5.3.1. Turn on the microcomputer system in the following order:
 - Interface/Power supply box (in-house design).
 - TRS-80 Keyboard.
 - TRS-80 Video Display.
 - TRS-80 Line Printer.
 - TRS-80 Expansion Interface.
- 5.3.2. When MEMORY? shows on the screen, press ENTER on the keyboard and READY> will appear. If the MEMORY? or READY> fails to appear hold the BREAK key and press the RESET button on the back of the keyboard.
- 5.3.3. Load the appropriate program using the CTR 80 recorder, or if available, using the TRS-80 disc drive. Refer to the User's Manual for details regarding loading procedure.
- 5.3.4. Enter RUN and the appropriate data via the TRS-80 Keyboard as requested. When START? appears, press ENTER to start the titration.

Preparation of the PET 2001 Microcomputer System

- 5.3.5. Turn on the microcomputer system in the following order:
 - Interface/power supply box (in-house design)
 - PET Keyboard/Video
 - PET Line Printer
 - PET Disc Drive
- 5.3.6. Load the appropriate program via the disc drive. Refer to the User's Manual for details regarding loading procedure.

- 5.3.7. Once the program is loaded type in RUN and press RETURN. Enter the appropriate data as requested on the screen, pressing RETURN after each entry.

5.3.8. **Comments on use of both microprocessors**

To interrupt execution at any time, use the BREAK key on TRS-80 or SHIFT and RUN/STOP keys on PET. To resume operation enter RUN then enter requested parameters.

If, on the PET, RETURN is hit without an input character, program execution is halted. To continue enter CONT.

There is no need to re-load the program on either system unless the power has been turned off.

A maximum of 150 data points are permitted for each titration.

The programs calculate inflection points during the titration. These appear on the display designated by an asterisk (*). These points are interpolated on the titration curve and, therefore, pH and volume appear "out of order" with respect to the remainder of the points. The inflection points are not printed in the titration table.

5.4. **Titration of samples for IP alkalinity.**

- 5.4.1. Pipette an appropriate volume of sample (usually 100 ml) into a clean beaker containing a stirring bar and place on a stirrer. Set stirrer at a rate which does not create a vortex.
- 5.4.2. Lower the electrode assembly into the sample ensuring that the electrode tip is clear of the stirring bar. In order to achieve pH stability it is necessary to adjust the relative heights of the delivery tip and pH electrode. Place the delivery tip as close as possible to the stirring bar and keep the electrode near the surface. Ensure that the porous plug of the electrode is below the surface of the sample.
- 5.4.3. After each titration is complete, press N for new sample or P for page feed and reply Y(yes) or N(no) for the same or new conditions when requested. Press BREAK on the TRS-80 or SHIFT and RUN/STOP on the PET when all samples are complete.
- 5.4.4. Power-off the equipment in the reverse order to the power-on sequence described in section 5.3.1. or 5.3.5.
- 5.4.5. Replace the rubber band over the filling hole of the electrode and store the electrode in the solution recommended by the manufacturer (distilled water for Ingold electrodes or pH 7 buffer for Radiometer electrodes).

6. **Calculation and Reporting**

The microcomputer Line Printer gives a hard copy of the complete titration table and data evaluation. The Gran IP alkalinity is reported to the nearest 0.01 mg/l alkalinity as calcium carbonate for values between -10 and +10 mg/l and to 3 significant figures for all other results.

7. Precision and Accuracy

The automated potentiometric IP alkalinity method exhibits a standard deviation of 0.05 mg/l calcium carbonate in the 0 - 10 mg/l calcium carbonate range. Some sample duplicates may deviate from this criterion due to the presence of particulate matter. Control limits have not been established.

8. Bibliography

- 8.1 Gran, G. (1952). Determination of the equivalence point in potentiometric titrations. Part II. *Analyst* 77: 661 - 671.
- 8.2 Ontario Ministry of the Environment (1979). Determination of the Susceptibility to Acidification of Poorly Buffered Surface Waters. Ontario Ministry of the Environment, Water Resources Branch.
- 8.3 Stumm, W. and Morgan, J.J. (1970). *Aquatic Chemistry*. Wiley-Interscience, New York, 583 p.
- 8.4. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1980). *Standard Methods for the Examination of Water and Wastewater*. 15th ed. APHA, Washington, D.C. 249-253.

THE DETERMINATION OF ANIONIC SURFACTANTS

(Methylene Blue Active Substances)

Methylene blue active substances (MBAS), such as alkyl benzene sulphonates (ABS), linear alkylate sulphonates (LAS), and alkyl sulphates are constituents of synthetic detergents. The surface-active properties of these anionic surfactants although ideal for cleaning purposes, have the objectionable tendency of causing persistent foam at sewage treatment plants and in receiving surface waters. This excess foam reduces the efficiency of normal oxygen replenishment at the surface of the water and has an undesirable appearance.

Ministry guidelines state that ABS concentrations should not exceed 0.5 mg/l in drinking water supply. Aquatic life may also be affected by the toxic properties of these surfactants. Both ABS and LAS cause similar problems and therefore waters and sewage are tested for these substances. The major difference between the two types of compounds is their rate of decay in the environment. ABS is not very biodegradable, while LAS is readily degradable. As a result, LAS is broken down in the sewage treatment plant and unlike ABS does not cause foaming problems in the receiving stream. Consequently, ABS has now been largely replaced in detergents by biodegradable surfactants.

Sample Handling and Preservation

Water, Sewage, Industrial Waste

As most synthetic detergents contain biodegradable anionic surfactants, samples must be refrigerated and tested immediately. When delays are unavoidable, samples should be preserved by acidification with sulphuric acid to a pH of less than 6.3 and refrigerated.

Selection of Method

For most water samples, the methylene blue method is quite satisfactory. This technique is only applicable for the determination of anionic detergents. Commercial detergents containing cationic or nonionic surfactants are also used and if their presence is suspected, specialized techniques have to be employed.

A special infrared technique has been developed for samples containing large amounts of interfering substances. This method, however, is not employed in the Ministry laboratories.

ANIONIC SURFACTANTS

Methylene Blue - Spectrophotometric Method A

SUMMARY

Matrix.	This method is routinely used on water, sewage and industrial waste samples.
Substance determined.	Anionic surfactants (methylene blue active substances, MBAS), primarily alkyl benzene sulphonates (ABS) and linear alkylate sulphonates (LAS).
Interpretation of results.	MBAS results are reported as the LAS equivalent in mg/l. Concentrations below 0.5 mg/l are not considered significant in a water supply.
Principle of method.	Methylene blue reacts with most anionic surfactants to produce blue colored salts which are extracted from water by chloroform. The intensity of the blue color is measured on a spectrophotometer, and compared to that produced by LAS.
Time required for analysis.	A single analysis requires about 30 minutes. Thirty-six samples may be tested in one day when performed concurrently by one person.
Range of application.	0.1 to 10.0 mg/l. Higher concentrations are determined on prediluted samples.
Standard deviation.	0.061 in the <2.0 mg/l MBAS range, 0.107 in the 2.0 - 5.0 mg/l MBAS range, and 0.151 in the 5.0 to 10.0 mg/l MBAS range for sewage and industrial waste.
Accuracy.	The average recovery of quality control solutions is 100.3%.
Detection criteria.	0.100 mg/l MBAS as LAS.
Interferences and shortcomings.	Unfortunately, numerous materials present in sewage and industrial wastes can interfere with the determination and lead to incorrect results and conclusions. Both organic and inorganic compounds form blue colored complexes with methylene blue in chloroform causing positive results. Organic amines complex with MBAS giving low results. Time consuming methods are available for removing these interferences but they are not routinely employed (8.2).

**Minimum volume
of sample.**

100 ml.

**Preservation and
sample container.**

Glass bottles are recommended. Most methylene blue active substances are biodegradable requiring that the sample be refrigerated and tested without delay. When delays are unavoidable, preserve by acidification with sulphuric acid to a pH of less than 6.3.

**Safety
considerations.**

Test should be conducted in a fume hood to protect operator from chloroform fumes.

ANIONIC SURFACTANTS

Methylene Blue - Spectrophotometric Method A

1. Introduction

Methylene blue reacts with LAS and most other anionic surfactants to produce a blue-colored salt which is readily extracted by chloroform from the original aqueous sample. The intensity of the blue color produced is proportional to the combined concentration of all methylene blue active substances present in the sample. Absorbances are measured at a fixed wavelength of 652 nm on a spectrophotometer. A calibration curve is obtained by plotting a series of known LAS standards against their respective absorbances. All results for methylene blue active substances are reported as their LAS equivalent.

2. Interferences and Shortcomings

Other organic and some inorganic compounds produce blue colored salts with methylene blue and cause positive interference. Inorganic cyanates, chlorides, nitrates and thiocyanates form blue-colored ion pairs with methylene blue and also cause positive interference. Negative interference is caused by amines which compete with methylene blue for surfactant sites.

NOTE: Due to time constraints no routine procedure is used to remove or suppress these interferences; however, methods are available in the literature (8.2).

3. Apparatus

- 3.1. Spectrophotometer with 2 cm cell for use at 652 nm.
- 3.2. Separatory funnels, 250 ml, preferably with Teflon stopcocks (48), and ground glass stoppers.
- 3.3. Volumetric flasks, 50 ml (24), 100 ml (5), 1000 ml (2).
- 3.4. Long stemmed funnels (24).
- 3.5. Graduated cylinders, 100 ml (2).
- 3.6. Reagent bottles, bulk storage, ground glass stoppers, 6 liter (2).
- 3.7. Glass wool.
- 3.8. Balance, analytical, weighing to 0.0001 g.
- 3.9. Chloroform distillation assembly, 3 liter capacity.

- 3.10 Ring stands with 2 circular funnel holders per stand arranged one above the other (4).
- 3.11. Mechanical shaker designed to hold separatory funnels (in-house design).

4. Reagents

- 4.1. LAS reference standard, prepared by the Soap and Detergent Association and issued as an analytical control by the U.S. Environmental Protection Agency. The standard is supplied in a sealed glass ampoule, and the active LAS content (w/w) is specified.

NOTE: Protect from light and store under refrigeration, but warm to 20°C before use.

- 4.2. Phenolphthalein, reagent grade powder.
- 4.3. Sodium hydroxide (NaOH), reagent grade pellets.
- 4.4. Monosodium dihydrogen phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$), reagent grade crystals.
- 4.5. Methylene blue, reagent grade powder.
- 4.6. Chloroform (CHCl_3), reagent grade.
- 4.7. Sulphuric acid (H_2SO_4), reagent grade, concentrated.
- 4.8. Nitric acid (HNO_3), reagent grade, concentrated.
- 4.9. **Sodium Hydroxide Solution (1 N)**

Dissolve 4.0 g sodium hydroxide pellets in distilled water and dilute to 100 ml. Safety precautions must be observed in handling sodium hydroxide, a very caustic chemical.

- 4.10. **Sulphuric Acid (1 N)**

Dilute 2.8 ml concentrated sulphuric acid to 100 ml with distilled water. Safety precautions must be observed in handling concentrated sulphuric acid.

- 4.11. **Phenolphthalein Indicator Solution**

Dissolve 1.0 g phenolphthalein powder in 100 ml 95% ethanol and mix with 100 ml distilled water.

- 4.12. **Methylene Blue Reagent**

Dissolve 0.50 g methylene blue powder, 250 g monosodium dihydrogen phosphate monohydrate, and 34 ml concentrated sulphuric acid in distilled water and dilute to 5.0 liters.

NOTE: Wear eye protection when handling concentrated sulphuric acid, and observe all safety rules pertaining to the use of concentrated acids.

4.13. Wash Solution

Dissolve 250 g monosodium dihydrogen phosphate monohydrate and 34 ml concentrated sulphuric acid in distilled water and dilute to 5.0 liters.

NOTE: Wear eye protection, and observe all safety rules pertaining to the use of concentrated acids.

4.14. LAS Stock Standard Solution (1000 mg/l)

The active LAS content of the solution in the glass ampoules is specified as a percentage (w/w), and ranges from 4.5 to 5.0%, e.g., 4.799%. Due to foaming problems, preparation of the stock standard is complicated. Using EPA data, prepare a 2000 ± 10 mg/l LAS solution by weighing to four-decimal places a suitable quantity of the standard in the ampoule, and diluting it with distilled water to 500 ml in a volumetric flask; calculate the actual LAS concentration. Dilute this solution to obtain a 1000 mg/l LAS standard, i.e., calculate the required volume of distilled water assuming there is no change in volume due to mixing. Use a volumetric flask and graduated pipettes.

NOTE: Shelf life for the stock solution is approximately six months.

4.15. LAS Working Standard (10 mg/l LAS)

Dilute 10 ml of stock LAS solution to 1 liter.

4.16. Quality Control Solutions

One quality control solution is prepared by diluting 10 ml of stock LAS solution to 1 liter with distilled water. Two levels (3.00 ml and 6.00 ml) of this solution and one blank are analyzed daily. The quality control solution is not stable and deteriorates within two months.

5. Procedure

- 5.1. Develop a calibration curve on a working LAS standard (10 mg/l LAS) whenever a new stock LAS standard (1000 mg/l LAS) is prepared. Differences between new and old calibrations should be minimal, e.g., 0.40 mg/l LAS standard should produce an absorbance value between 0.38 and 0.42 units for the specified experimental conditions.

- 5.1.1. Prepare a blank by using 100 ml distilled water.

- 5.1.2. Prepare a series of standards from LAS working solution (10 mg/l LAS) using 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml and 6.0 ml aliquots in separate separatory funnels. Dilute with distilled water to a final volume of 100 ml. This gives a series of standards with concentrations 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/l LAS.

- 5.1.3. Carry the blank and standards through procedures 5.2 to 5.20. and prepare a calibration curve by plotting the absorbance of each standard versus its respective concentration. A linear curve conforming to Beer's law should be obtained.

- 5.2. Use concentrated nitric acid to wash all glassware and then rinse with distilled water.

NOTE: WEAR EYE AND HAND PROTECTION.

- 5.3. Check to make sure that all stopcocks on the separatory funnels are closed.
- 5.4. Prepare a blank by adding 100 ml distilled water to a separatory funnel.
- 5.5. Prepare QC-A and QC-B solutions for analysis by adding 3.00 ml and 6.00 ml of working LAS standard (10 mg/l LAS) to separatory funnels. Dilute to 100 ml by adding distilled water.
- 5.6. Shake the sample to be tested and pipette an aliquot ranging between 5.0 and 100 ml into a separatory funnel. An estimate of the dilution required is obtained from the amount of frothing that occurs upon shaking the sample vigorously. If no frothing occurs, the LAS concentration is less than 0.1 mg/l.
- 5.7. Add distilled water until the total volume is approximately 100 ml.
NOTE: Use the following procedure for all blanks, quality control solutions and samples.
- 5.8. Add 3-4 drops phenolphthalein indicator.
- 5.9. Add 1 N sodium hydroxide dropwise while swirling until the solution just turns pink.
- 5.10. Add 1 N sulphuric acid while swirling until the pink color just disappears.
- 5.11. Add 25 ml of methylene blue reagent and shake well.
- 5.12. Add 10 ml of chloroform, stopper and shake well for about 30 seconds.
NOTE: Pressure rapidly builds up in the separatory funnel and must be released at frequent intervals by opening the stopcock. Avoid excessive inhalation of chloroform vapors.
- 5.13. Allow the phases to separate. If an emulsion forms, it may be broken down by rapidly spinning a piece of wire at the interface of the solvent layers.
- 5.14. Draw off the chloroform layer into a second separatory funnel containing 50 ml of wash solution.
- 5.15. Repeat the extraction twice, using 10 ml of chloroform each time. Combine all extracts in the second separatory funnel. If the blue color in the aqueous phase becomes faint or disappears, add an additional 25 ml methylene blue reagent.
- 5.16. Shake the combined extracts with the wash solution for about 30 seconds, allow the phases to separate, and filter the chloroform layer into a 50 ml volumetric flask through a long stemmed funnel containing glass wool.
- 5.17. Add another 8-9 ml of chloroform to the wash solution, shake well, allow phases to separate and filter through the same glass wool into the same 50 ml volumetric flask. Repeat this step once more.
- 5.18. Rinse the glass wool with chloroform while adjusting the final volume to exactly 50 ml. Stopper and mix well by inverting several times.

5.19. At a fixed wavelength of 652 nm, use the extracted distilled water blank to zero the spectrophotometer.

5.20. Record the absorbance of the chloroform extracts at 652 nm.

NOTE: The chloroform is recycled by distilling all extracts, discarding the first 50 ml of distillate. The distilled chloroform is then used for additional analyses.

6. Calculation and Reporting

The amount of methylene blue active substances (MBAS) as LAS in the chloroform extract is calculated from known absorbance values using the previously constructed calibration curve. The following calculation is then used to determine the amount of MBAS as LAS in the original sample.

$$\text{mg/l MBAS as LAS} = \frac{a}{v} \times 100$$

Where:

a = mg/l LAS from calibration curve

v = sample aliquot in ml

Final results are rounded off and reported according to the following table.

<u>MBAS as LAS (mg/l)</u>	<u>Reported to nearest (mg/l)</u>
0.0 - 4.9	0.1
5.0 - 9.9	0.2
10.0 - 19.5	0.5
20 - 40	1
41 - 70	2

7. Precision and Accuracy

Standard deviations of duplicate samples were 0.061 in the 0-2 mg/l range, 0.107 in the 2-5 mg/l range and 0.151 in the 5-10 mg/l MBAS range. The average recovery of quality control solutions is 100.3% with relative standard deviations ranging from 1.65 to 2.20%.

8. Bibliography

8.1. ABCM-SAC Joint Committee (1957) Method for the determination of synthetic detergents. Analyst, 82: 826-834.

- 8.2. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971) Standard Methods for the Examination of Water and Wastewater, 13th ed., APHA, Washington, D.C., 600-603.
- 8.3. Degens, P. N., Evans, H. C., Kommer, J. D. and Winsor, P. A. (1953). Determination of sulphate and sulphonate anion-active detergents in sewage. *Journal of Applied Chemistry*, London, **3**: 54-61.
- 8.4. Degens, P. N., Van der Zee, H., and Komer, J. D. (1953). Routine method for determination of sulphate and sulphonate anion-active detergents in sewage. *Sewage and Industrial Wastes*, **25**: 24-25.
- 8.5. Evans, H. C. (1950) Determination of anionic synthetic detergents in sewage. *Journal of the Society of Chemical Industry*, Supplementary Issue No. 2, **69**: 576 - 580.
- 8.6. Jones, J. H. (1945). Colorimetric method for determination of small quantities of sulphonated or sulphated surface-active compounds. *Journal of the Association of Official Agricultural Chemists*, **28**: 398-409.
- 8.7. Kay, S. E. (1950). "Evans" modified methylene blue method for the estimation of anionic detergents in sewage. *Institute of Sewage Purification Journal and Proceedings*, **4**: 403-407.
- 8.8. Klein, L. (1959) *River Pollution 1: Chemical Analysis*, Butterworth and Co. Ltd., London, 206 p.
- 8.9. Lester, W. F. and Raybould, R. D. (1950) Determination of anionic-active detergents in sewage and effluents. *Institute of Sewage Purification Journal and Proceedings*, **4**: 392 - 402.
- 8.10. Longwell, J. and Maniece, W. D. (1955) Determination of anionic detergents in sewage, sewage effluents and river waters. *Analyst*, **80**: 167-171.

THE DETERMINATION OF ARSENIC

Arsenic is widely distributed in the natural environment, occurring in small amounts in soils, waters and plants. Weathering of the widespread mineral arsenopyrite (AsFeS) releases arsenic to soil, water and biological systems.

Arsenic and its compounds can be toxic to plants, animals and humans. Arsenical dust may cause dermatitis, bronchitis and irritation to the upper respiratory tract. As a gas, organic arsenic can cause severe eye damage. Ingestion of arsenic can result in stomach and intestinal upsets and can produce keratosis and skin cancer. At high intake levels, particularly in the organic form, arsenic accumulates in the human body, concentrating in the skin, nails and hair.

Man contributes arsenic to the environment through insecticide, herbicide and pesticide use, coal utilization, petroleum production and arsenical ore smelting operations. Arsenic salts are used in the manufacture of glassware and ceramics, tanning and in some chemical manufacturing processes. In other nations, arsenic is used as a desiccant for cotton prior to machine picking. Arsanilic acid when used as an additive in animal feeds is known to improve the health, appearance, survival and production efficiency. Shortly before slaughter arsanilic acid should be removed from the animal's diet to allow it to clear from the system.

Natural arsenic concentrations seldom exceed $1 - 2 \mu\text{g/g}$ in dry plant tissue, and $1 - 8 \mu\text{g/l}$ in the aquatic environment. The maximum permissible level of arsenic in domestic water supplies is $15 \mu\text{g/l}$. Ambient air contains up to $0.005 \mu\text{g/m}^3$ of arsenic over a 24 hour period. The maximum ambient air quality criteria for arsenic is $25 \mu\text{g/m}^3$ over a 24 hour period and is currently under review.

Sample Handling and Preservation

Water, Sewage and Industrial Waste

A minimum volume of 50 ml is required for analysis. Plastic or glass bottles with plastic lined caps may be used. Nitric acid preservative (1 ml per liter) is required. Samples can be stored for up to one month without loss of arsenic.

Soil and Sediment

Soil samples are normally collected in conjunction with vegetation samples in order to differentiate between current and past emission situations. Occasionally, soil samples are collected to establish background conditions. The soil is sampled with a 2 cm diameter stainless steel tube. A minimum of 10 cores are taken from each sampling site. Each core is fractionated into depths of 0 - 5 cm, 5 - 10 cm, and 10 - 15 cm, and each level placed in an appropriately labelled plastic bag for shipping.

Vegetation

To ensure correct interpretation of analytical data, all samples being compared must be carefully matched according to plant species, age or maturity of leaf tissues, age of tree or shrub, and position of sample on tree or shrub. Usually, foliage is collected from that side of the tree or shrub facing the presumed source of air pollution, but occasionally, a second sample is taken from the side opposite to the source. Samples are taken by trimming outside growth from ground level up to twenty feet or more and collecting all leaves. This provides a 50 - 100 g composite sample of fresh material. Samples are placed in perforated polyethylene bags and refrigerated. Forage samples (grass) are collected by cutting the terminal 25 cm of stems and blades over the representative area and then sampling at ten step intervals. Dried flower heads and stalks are discarded and no root material is included.

All vegetation samples as collected are potentially unstable, and will decompose unless properly handled. Vegetation samples can be preserved for a few weeks under refrigeration, and when dried at 80°C for 30 hours in a forced air draft oven, will become almost permanently stable.

Air Particulate

Hi-Vol glass fibre filters are used to sample air particulate matter. Air is drawn through the covered housing at 40 - 60 cfm and through a tared glass fibre filter paper. Suspended particles with an aerodynamic diameter of less than 100 $m\mu$ can pass through the filter. The unit operates on a timed 24 hour cycle. After each sampling period the exposed filter is removed, folded across the middle of the 10 inch side, placed in an envelope and mailed to the laboratory. The sample must be kept dry at all times.

Selection of Method

An automated method is employed which uses arsine gas generation followed by atomization in a heated open ended quartz tube and measurement by atomic absorption spectroscopy. If total arsenic is to be determined a predigestion procedure is required which varies with sample matrix. Samples are digested with nitric, perchloric and sulphuric acid. The digestion procedure is not required for soluble arsenic.

TOTAL ARSENIC

Hydride Generation - Atomic Absorption Method A

SUMMARY

Matrix	This method is used for arsenic determinations on water, sewage, biomaterials, industrial effluent, sludge, soil, sediment, vegetation and Hi-Vol filter samples.
Substance determined.	Arsenic as As III.
Interpretation of results	Results are reported as total arsenic in mg/l.
Principle of method.	Samples and standards are digested in oxidizing acid mixtures to convert all forms of arsenic to arsenate ion, AsO_4^{3-} . In an automated system the arsenate in acid solution is reduced by sodium borohydride to arsine which is swept into a heated quartz tube by argon carrier gas. The gases in the tube spontaneously form a hydrogen-argon, entrained air flame which reduces the arsine to atomic arsenic which is determined by conventional atomic absorption technique.
Time required for analysis.	The automated system analyzes 25 acid digests/hr. If sample preparation, dilution and calculation time is included, one person can analyze 40 - 50 samples per day.
Range of application	Usually 1 - 40 $\mu\text{g/l}$. Dilution can be used to extend the range.
Standard deviation.	Varies slightly depending on sample matrix, see Table 2.
Accuracy.	NBS Orchard Leaves, Bovine Liver and EPA 1171 and 575 Waters were analyzed and the certificate value obtained within 5%. Control samples A and B (see Table 1) are not allowed to vary by more than 2 standard deviations from the expected A and B value.
Detection criteria.	The normal working detection limit is 1.0 $\mu\text{g/l}$. A detection limit of 0.2 $\mu\text{g/l}$ can be obtained using scale expansion.
Interferences and shortcomings.	High concentrations of selenium, antimony, cobalt, nickel and tin cause depression of the signal. These elements are rarely found in high enough concentrations in environmental samples to cause significant depression of the signal.

**Minimum
sample size.**

50 ml of solution or 0.5 g of solid is sufficient.

**Preservation and
sample container.**

Glass or plastic bottles are suitable for water, sewage and industrial wastes. Water and sewage samples should be preserved by acidifying with nitric acid (1 ml per liter). Solid samples may be dried and ground and stored in jars.

**Safety
considerations.**

Arsenic compounds are very toxic and should be handled with utmost care. Arsine gas is poisonous and therefore analyses must be carried out in well ventilated areas.

TOTAL ARSENIC

Hydride Generation - Atomic Absorption Method A

1. Introduction

A sample aliquot is digested with a strong oxidizing acid to convert all forms of arsenic to arsenate. Arsenate is then reduced to arsine gas by sodium borohydride. The arsine gas is swept by a carrier gas into a heated quartz tube, where the mixture of air, argon, hydrogen and arsine spontaneously ignites forming an argon hydrogen air flame reducing the arsine gas to arsenic atoms which are measured by atomic absorption spectroscopy.

2. Interferences and Shortcomings

The technique is relatively free of interferences. High concentrations of other elements which also react with sodium borohydride, such as selenium, antimony, cobalt, nickel and tin, will cause depression of the signal. These elements, however, are rarely found at high enough concentrations to cause problems.

3. Apparatus

- 3.1. Cork borer #11 (Hi-Vol sample Cutter).
- 3.2. Test tubes 18 x 150 mm, calibrated to 15 ml.
- 3.3. Aluminum heating block with 40 to 60 holes for accommodating test tubes.
- 3.4. Hot plate with heat control rheostat.
- 3.5. Automatic sampler, Technicon or equivalent.
- 3.6. Proportioning pump, Technicon or equivalent.
- 3.7. Glassware and mixing coils, Technicon or equivalent. Assemble sampler, proportioning pump, gas liquid separator and atomic absorption spectrophotometer as shown in Figures 1 and 3.
- 3.8. Heated quartz tube (a heated and insulated quartz cell as shown in Figure 2).
- 3.9. Voltage regulator.
- 3.10. Atomic absorption spectrophotometer with arsenic hollow cathode lamp (or electrodeless discharge lamp).
- 3.11. Recorder (or data output device).

4. Reagents

4.1. Sulphuric acid (H_2SO_4) concentrated, reagent grade.

4.2. Arsenic trioxide (As_2O_3), reagent grade.

4.3. Argon gas.

4.4. Nitric acid (HNO_3) concentrated, reagent grade.

4.5. Hydrochloric acid (HCl) concentrated, reagent grade.

4.6. Sodium borohydride (NaBH_4) (98% powder).

4.7. Sodium hydroxide (NaOH) (reagent grade, pellets).

4.8. Sodium Borohydride Solution (2% w/v)

Dissolve 10 g of 98% sodium borohydride in 200 ml distilled water in a graduated 500 ml Erlenmeyer flask. Add 2 or 3 pellets of sodium hydroxide and bring the final volume to 500 ml. Prepare fresh daily.

4.9. Potassium Iodide (10% w/v)

Dissolve 20 g potassium iodide in 200 ml distilled water.

4.10. Arsenic Stock Solution (1000 mg/l As)

Dissolve 1.3202 g arsenic trioxide in a minimum volume of 20% sodium hydroxide. Transfer quantitatively to a one liter volumetric flask, neutralize excess caustic with hydrochloric acid and bring to the mark with distilled water.

4.11. Perchloric acid (HClO_4) concentrated, reagent grade.

4.12. Standard Arsenic Solution (3 mg/l As)

Dilute 3 ml of stock arsenic solution to 1000 ml with 5% hydrochloric acid.

4.13. Arsenic Calibration Standards

Digested standards are prepared from the standard arsenic solution. Recommended sample concentrations are given for each sample type in Table 1.

NOTE: If selenium is also to be measured combined standards can be prepared. (SEE DETERMINATION OF SELENIUM.)

4.14. Quality Control Solutions

For Hi-Vol, vegetation, soil, sediment and sludge samples calibration control solutions are prepared by judicious blending of at least 1 month's supply of sample digestates to provide controls as QC-A in the 10% - 20% and QC-B in the 80% - 90% range of instrumental response.

For water samples calibration control solutions are prepared by diluting 5 and 10 ml of standard arsenic control solution as in 4.12, diluted to 1 liter with tap water.

For vegetation and water sample, 2 reference samples are used as quality control samples.

4.15. Sensitivity Checks

An undigested 20 µg/l sensitivity check is prepared from the standard arsenic solution.

5. Procedure

5.1. Sample Digestion and Quality Control

5.1.1. Quality Control

For all sample types, spike additions are made to every 20th sample with 0.05 ml of stock standard solution as in 4.12 for accuracy control of matrix effects.

Every 19th sample is introduced as a duplicate of the preceding sample for precision control.

For vegetation and water sample, 2 reference samples are digested with each batch of sample for control of sample preparation, recovery and accuracy check.

5.1.2. Water and Industrial Effluent

5.1.2.1. Pour an appropriate aliquot (usually 15 ml) of sample into a 100 ml beaker.

5.1.2.2. Add 3 ml of a 6:3:1 nitric/perchloric acid mixture and place on a hot plate and heat until dense white fumes appear.

5.1.2.3. Cool samples, add 0.5 ml distilled water 2 ml concentrated HCl, and make to 15 ml with distilled water, mix well.

5.1.2.4. Prepare a digested set of standards as in Table 1 as well as 2 synthetic control samples. Analyze samples and standards according to 5.2.

NOTE: Every 18th sample is analyzed in triplicate with one aliquot being spiked. Analyze samples within 2 days of preparation since the valence state of the arsenic may change affecting instrumental response.

5.1.3. Sludge and Sewage

5.1.3.1. Pipette 1 ml of a well mixed sample into a test tube calibrated at 15 ml.

5.1.3.2. Add 3 ml of a 6:3:1 mixture of sulphuric:nitric:perchloric acid and heat until white fumes appear. Prepare standards as in Table 1.

5.1.3.3. Cool tubes, add 0.5 ml distilled water and 2 ml HCl, bring to 15 ml with distilled water, mix well.

5.1.4. Biomaterials

- 5.1.4.1. Weigh an appropriate aliquot (usually 2 g) of tissue into a 50 ml test tube. Add 5 ml concentrated nitric and 2 ml concentrated perchloric acid and heat tubes at 170°C until heavy white fumes appear.

NOTE: Hot concentrated perchloric acid can be explosive. As acid volume decreases watch samples closely. If darkening occurs add an additional 2 ml of concentrated nitric acid immediately.

- 5.1.4.2. When 1 - 2 ml of a pale colorless or pale yellow solution remains, cool tubes, rinse down with 10 ml distilled water and bring to boiling. Cool tubes and add 25 ml distilled water. Analyze according to 5.2. Prepare standards as in Table 1.

- 5.1.4.3. Smaller aliquots (\approx 0.2 g) can be digested as in 5.1.2.

5.1.5. Soil, Sediments and Vegetation.

- 5.1.5.1. Weigh a .06 g sample using weighing paper. Transfer to a 18 x 150 mm glass test tube calibrated at 15 ml. Include 0, 10, 20, 30 and 40 μ g/l standards and 1 control sample with each run.

- 5.1.5.2. Place tubes in a 40-hole aluminum block and add 3 ml of a 6:3:1 sulphuric:nitric:perchloric acid mixture.

- 5.1.5.3. Transfer block to cold hot plate. Set hot plate to 110°C and heat samples. Digest for a minimum of 6 hours and preferably overnight, until white fumes appear.

- 5.1.5.4. Cool tubes, add 0.5 ml distilled water and 2 ml HCl, bring to 15 ml with distilled water, mix well.

NOTE: A clean solution with a slight yellowish tinge and a few white silica particles is obtained from vegetation samples. In soil and sediment particles more undissolved siliceous material remains.

NOTE: Analysis of these digests gave results comparable to those of alkaline fusion and acid extraction soil digests.

5.1.6. Air Particulate (Hi-Vol Filters)

- 5.1.6.1. Cut 2, 1.8 cm diameter circles from the exposed Hi-Vol filter and place in a 18 x 150 mm glass test tube calibrated at 15 ml. Add 3.0 ml of a 6:3:1 nitric:sulphuric:perchloric acid mixture and digest in an aluminum block overnight, until white fumes appear.

- 5.1.6.2. Cool, add 0.5 ml distilled water and 2 ml HCl, bring to the 15 ml mark with distilled water, mix well.

- 5.1.6.3. Digest standards (Table 1) in the presence of a filter blank.

5.2. Arsenic Determination

REFER TO ATOMIC ABSORPTION SPECTROPHOTOMETER MANUFACTURER'S MANUAL FOR RECOMMENDED SETTINGS FOR ARSENIC. SET UP AUTOANALYZER ACCORDING TO MANUFACTURER'S INSTRUCTION MANUAL.

5.2.1. Adjust quartz cell position for maximum transmittance. Set up system as in Figure 1.

5.2.2. Set up AutoAnalyzer. (See Figure 1).

NOTE: Allow 1 hour warm-up time for all systems. The cells are prepared by wrapping with chromel resistance wire (≈ 1 ohm/ft), and covering with asbestos string or similar insulation. Leads are connected to a Variac transformer. Proper cell temperature is found by trial and error.

5.2.4. See Table 1 for run formats, ranges and quality control procedures. All systems operate at a rate of 25 samples/hour with a 1:2 sample: wash ratio.

6. Calculation and Reporting

6.1. Water, Sewage, Industrial Effluent and Sludge

Results are calculated using a computer drawn standard curve constructed from the peak heights of the digested standards. The curve is a least squares fit constrained to pass through the digested blank. The calculated concentrations in solution are multiplied by the appropriate dilution factor and reported.

$$\text{As (mg/l)} = C \times \text{DF}$$

Where:

C = arsenic concentration in sample solution in mg/l

DF = dilution factor

6.2. Soil and Vegetation

The peak heights are measured and the arsenic concentrations in the digested solutions are determined, from a standard curve. For a .06 g sample which has been digested and brought to 15 ml:

$$\text{As (}\mu\text{g/g)} = C \times 250$$

Where:

C = arsenic concentration in solution in mg/l.

6.3. Hi-Vol Filters

Digest concentrations are calculated as above. Total arsenic in $\mu\text{g/m}^3$ is calculated as follows:

$$\text{As } (\mu\text{g}/\text{m}^3) = \frac{\text{C}(\text{DF}) \times 1.181}{\text{V}}$$

Where:

C = solution concentration in $\mu\text{g}/\text{l}$

DF = dilution factor

V = volume of air sampled in m^3

$$1.181 = \frac{e}{f} \times d = \frac{406.25}{5.16} \times 0.015$$

Where:

e = total exposed filter area

f = filter aliquot taken

d = volume of digestate/1000

Results are reported to 2 significant figures.

7. Precision and Accuracy

Standard deviations are presented in Table 2. Recovery on NBS orchard leaves and bovine livers and on EPA waters is $100\% \pm 5\%$. Control of A and B solutions is maintained to within 2 standard deviations of the expected A and B values.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th ed., APHA, Washington, D.C. 159-162, 283.
- 8.2. Vijan, P. N., Rayner, A.C., Sturgis, D. and Wood G.R. (1976). A semi-automated method for the determination of arsenic in soil and vegetation by gas-phase sampling and atomic absorption spectrophotometry. *Analytical Chemica Acta*, **82** (2): 329-336.
- 8.3. Vijan, P. N. and Wood, G.R. (1974). An automated submicrogram determination of arsenic in atmospheric particulate matter by flameless atomic absorption spectrophotometry. *Atomic Absorption Newsletter*, **13** (2): 33 - 37.

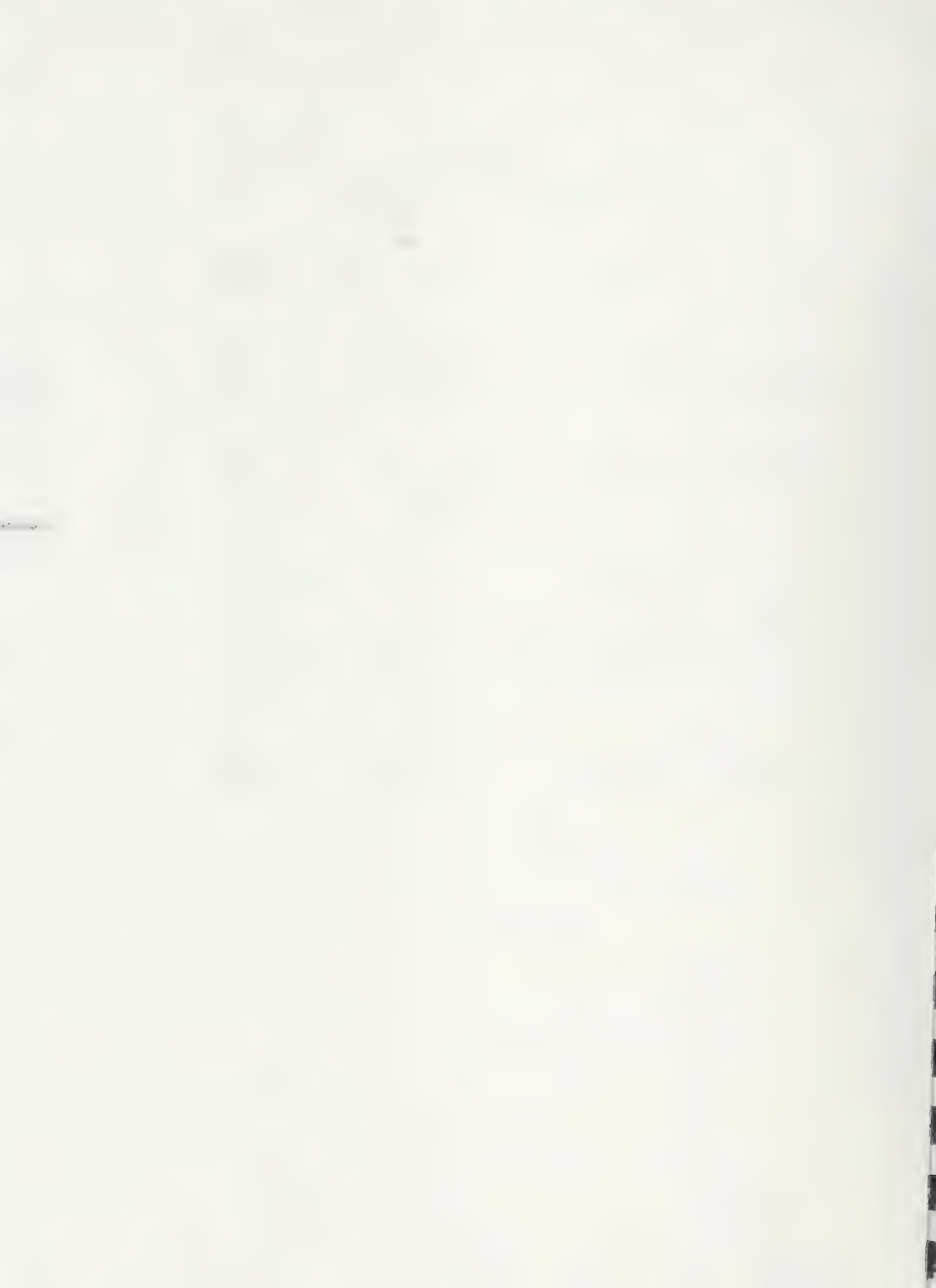
TABLE 1 Run Formats and Quality Control μ g/l

Matrix	Sensitivity Check	Sensitivity Absorbance Unit	Maximum Run Size	Prepared each run Run before and after samples	Digested Standards μ g/l	Quality Controls	Other
Water	Undigested 20μ g/l standard	0.15	150		0, 10, 20, 30, 40	A, B mixed metal aqueous standards	Every 18th sample in triplicate with one aliquot spiked
Sludge	Undigested 20μ g/l standard	"	80	"	0, 10, 20, 30, 40	"	"
Hi-Vol	Undigested 20μ g/l standard	"	"	"	0, 10, 20, 30, 40	A, B composite Hi-Vol digestate	"
Vegetation	Undigested 20μ g/l standard	"	"	"	0, 10, 20, 30, 40	A, B composite vegetation or soil digestate	"
Soil and Sediment	Undigested 20μ g/l standard	"	"	"	0, 10, 20, 30, 40	2 reference standards 0.1 g aliquots of each	"



TABLE 2
Precision Data for Arsenic Analysis

Sample Type	Duplicates	Concentration Range	Standard deviation
Waters and sludges	Within-run	0-0.020 mg/l	0.001
		0.02-0.050	0.004
		0.05-0.10	0.004
Sediments and soils	Within-run	0-5.0 µg/g	0.24
		5-10	0.49
		10-50	2.2
		50-100	5.7
		100-300	9.9
Sediments and soils	Between-run	2.4 µg/g	0.2
		3.0	0.3
Hi-Vol filters	Between-run	14 µg/m ³	1.0
		20	0.4
		16	2.0
Vegetation	Within-run	0-2.0 µg/g	0.06
		2.0-10.0	0.02



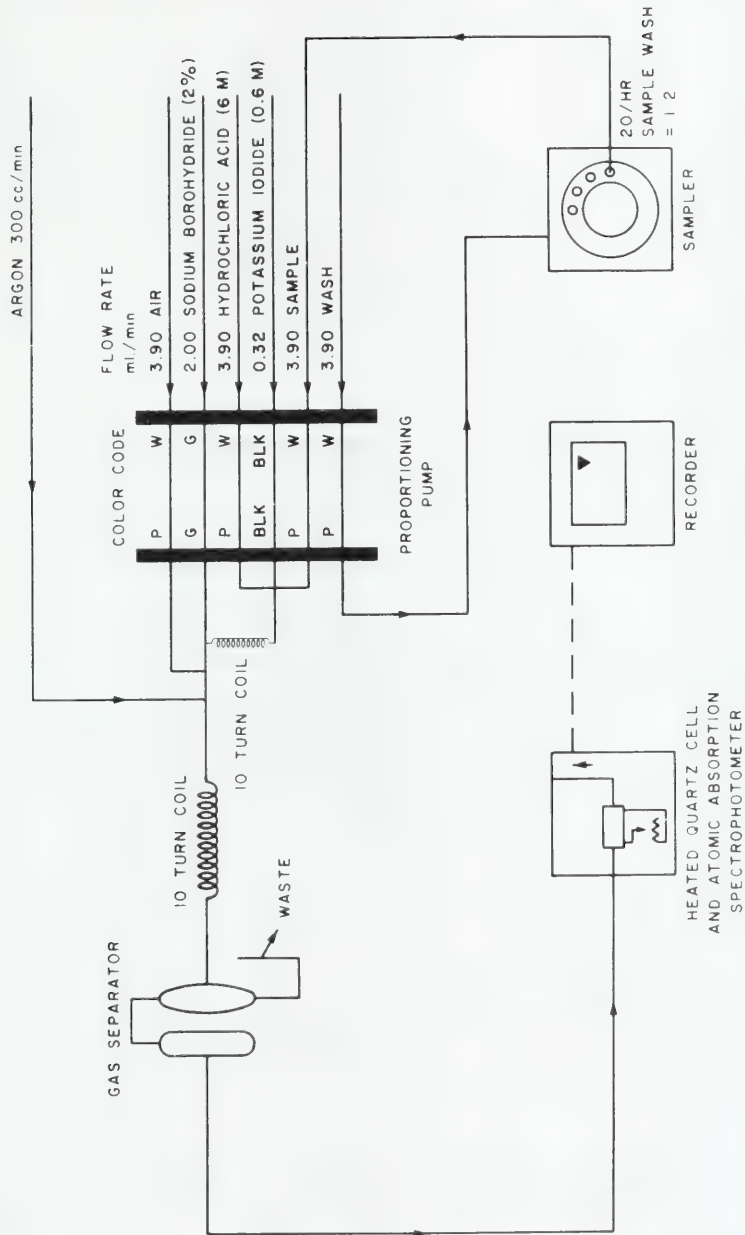


FIGURE 1 — FLAMELESS ATOMIC ABSORPTION MANIFOLD FOR ARSENIC DETERMINATION

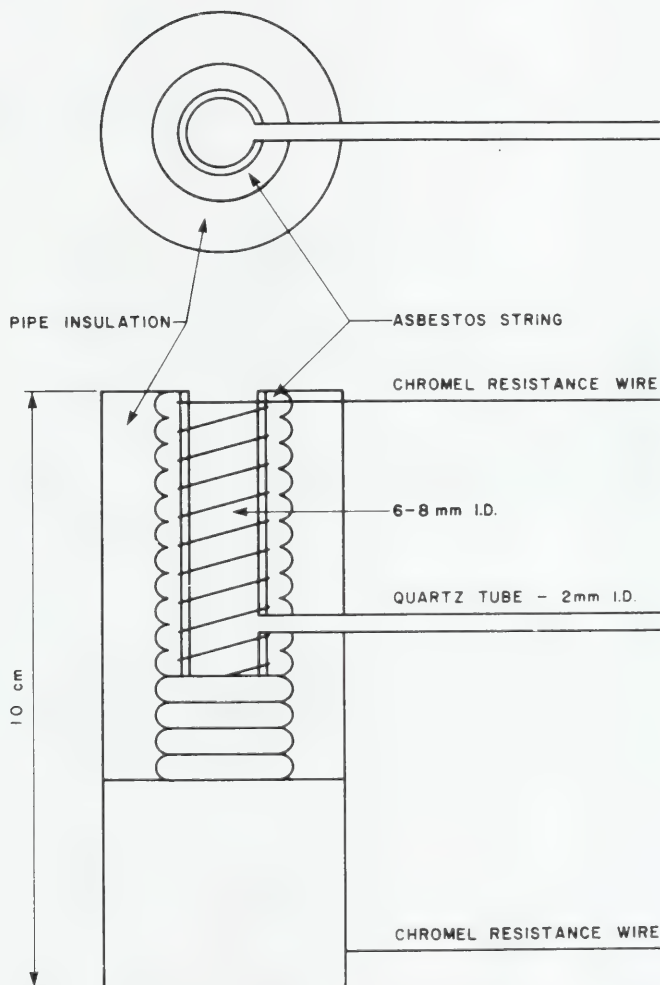


FIGURE 2 - QUARTZ TUBE FURNACE ASSEMBLY

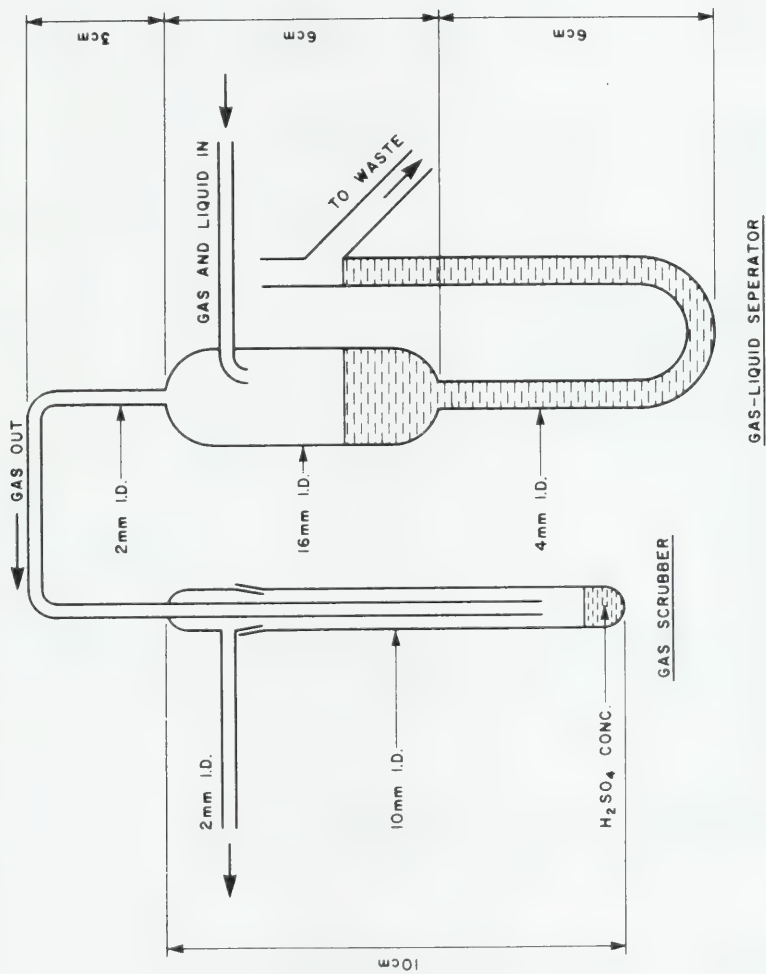


FIGURE 3 - IMPINGER AND GAS SEPARATOR ASSEMBLY

THE DETERMINATION OF ASBESTIFORM FIBRES

Asbestos is a generic term for several fibrous silicate minerals of the serpentine and amphibole group. Chrysotile or white asbestos is the sole species classified in the serpentine group, but it is by far the most abundant kind of asbestos. The amphibole group includes asbestiform types, namely crocidolite, amosite, anthophyllite, tremolite and actinolite.

Asbestos occurs as an environmental water and air pollutant through both natural causes and human activities. Although erosion of rock formations introduces fibres into bodies of water, modern usage of asbestos in over four thousand industrial and domestic applications has provoked concern over the amount introduced into the environment by the mining and milling of asbestos and the manufacturing and use of asbestos products, e.g. insulation, paint, brake linings, etc. Mining operations in asbestos bearing ore may also release asbestos into water supplies.

Cases of asbestosis in the asbestos industry have revealed an association between asbestos and severe lung damage with the likelihood of cancer developing following heavy exposures. Epidemiological evidence accumulated over the years indicates that asbestos fibres are found in the lungs of most city dwellers as well as in the air. However the effect of environmental levels of asbestos on the general population is not clear. Growing concern for long-term health effects of airborne asbestos has led to the belief that asbestos concentration in the atmospheric environment should be measured continuously and changes of its level carefully monitored.

Sample Handling and Preservation

Water Samples

A polyethylene bottle of 1 liter capacity, preferably new and fitted with a plastic screw cap, is rinsed at least three times with the water from which the sample is to be taken and then filled to one inch from the top of the bottle with the water sample. Samples should be shipped to the laboratory without delay and be protected from freezing.

All samples submitted to the laboratory for asbestos analysis must be accompanied by pertinent sampling data.

If the sample cannot be filtered within 48 hours after collection, it should be stored in a cool, dark location and 1 ml of a 2.71% mercuric chloride solution may be added per liter of sample by the laboratory to inhibit algal and bacterial growth.

Air Samples

The Hi-Vol sampler, fitted with a Nuclepore (20 cm x 25 cm) filter of 0.4 μ m pore size, is recommended for sampling airborne asbestos. The sampler should be equipped with a transducer and a continuous air flow recorder.

Due to the difficulty in handling the Nuclepore filter under field conditions, installation of the filter in a Hi-Vol cassette (or filter holder) is carried out in a sheltered environment. The cassette may then be attached to and removed from the Hi-Vol at the given field location. After removal of the filter from the cassette, the filter is placed on a 20 cm x 25 cm separator sheet (supplied with the filter) with the sample side facing up and both folded in half along the 20 cm width. The entire package is then placed in a glassine envelope and delivered to the laboratory together with the air flow chart and other pertinent information.

Selection of Method

There are several techniques and types of instrumentation that can be used for the analysis of asbestos. However, the small fibres usually encountered in environmental samples can only be resolved by means of an electron microscope.

Presently the transmission electron microscope (TEM) is the instrument of choice because it resolves very small fibres; it reveals the internal structure of fibres, which is valuable for chrysotile identification and it is capable of obtaining selected area electron diffraction (SAED) patterns of single fibres.

SAED is the preferred technique for routine identification of fibres and can be supplemented by energy-dispersive X-ray (EDX) analysis in samples requiring more rigorous identification. The routine use of SAED patterns as opposed to EDX analysis is justified by the fact that single crystals of minerals have characteristic SAED patterns whereas amphibole minerals may give X-ray spectra similar to minerals of other groups such as ferromagnesium sheet silicates.

Various methods of sample preparation and analysis by TEM were compared by a committee formed by this Ministry in 1976 to outline a method for the determination of asbestos fibres in water. The method for water samples recommended by the committee is the most precise and reproducible of the methods tested and is that described.

ASBESTIFORM FIBRES IN WATER

Using Transmission Electron Microscopy

SUMMARY

Matrix.	This method is used routinely for asbestos determination in water.
Substance determined.	The method determines the concentration of asbestos fibres in million fibres per liter (MFL) and gives an estimation of the mass concentration in micrograms per liter and an indication of the amounts of other fibres in water. The size distributions of the asbestos fibres can also be provided.
Interpretation of results.	<p>The reported concentration of fibres is expected to be lower than the actual concentration, especially for amphiboles, because a variable fraction of fibres may not give identifiable SAED patterns. In the case of amphiboles, an over-estimation of the mass concentration may be made because of the manner in which fibre volumes are calculated.</p> <p>The results reported are rounded to the nearest 0.2, 1 and 5 MFL for concentrations in the ranges of less than 1, 1 to 10, and 20 to 100 MFL respectively and to 2 significant figures for concentrations greater than 100 MFL for the following reasons:</p> <ol style="list-style-type: none">1. The accuracy of the method has not been directly investigated.2. The precision of the method, based on a recent inter-laboratory study was found to be 50% expressed as relative standard deviation.3. Blank asbestos levels are variable by as much as 0.2 MFL. <p>The 95% confidence levels given with the results are derived from the fibre counts per grid opening and therefore provide a measure of the precision of each analysis.</p>
Principle of method.	<p>The sample is filtered through a membrane filter to isolate the particulate matter, including asbestos, using a procedure designed to deposit the particles uniformly on the filter surface. A layer of carbon is evaporated onto the filter surface and sections of the coated filter are placed on transmission electron microscope grids. The filter is dissolved with chloroform, leaving the particulate matter embedded in a layer of carbon on the grids, which are then examined in a transmission electron microscope at a magnification of 20,000X.</p> <p>A representative area of the grid is examined and fibres in this area are counted, sized and classified to obtain size distributions of the fibres and the average number of fibres per unit area of the</p>

grid. The average number of fibres is converted to concentration in MFL and the average fibre size is used to calculate the mass concentration in micrograms per liter.

Amphibole fibres are identified by their SAED patterns and chrysotile fibres are identified by their tubular morphology with confirmation of some fibres as chrysotile by SAED.

Samples containing large amounts of insoluble organic material are subjected to filtration, ashing and ultrasonic treatment prior to final filtration.

**Time required
for analysis.**

24 hours for sample preparation (a batch of several samples may be prepared at one time); 3 hours for electron microscopic examination; 1 hour for calculations.

**Range of
application.**

A range of concentration of about 0.1 MFL to 1000 MFL can be determined without resorting to dilution of the original water sample.

**Standard
deviation.**

Within laboratory precision - approximately 25% expressed as relative standard deviation.

Accuracy.

Unavailable.

**Limit of
detection.**

Defined as the concentration corresponding to 1 fibre counted, typically 0.2 MFL.

**Interferences
and shortcomings.**

Presence of some non-asbestos fibres can result in inaccurate fibre counts. The sample preparation may cause losses of fibres or break-up of fibre bundles. Presence of particulate material may obscure fibres. Identification procedures have limitations.

**Minimum volume
of sample.**

5 ml.

**Preservation and
sample container.**

Sample container is a 1 liter polyethylene bottle, preferably new and fitted with a plastic screw cap. Mercuric chloride solution may be added at the laboratory if a sample cannot be filtered within 48 hours of sampling.

**Safety
considerations.**

Normal safety precautions.

ASBESTIFORM FIBRES IN AIR

Transmission Electron Microscopy

SUMMARY

Matrix.	This method is used routinely for asbestos determination in air.
Substance determined.	The method determines the concentration of asbestos fibres in fibres per cubic centimeter and gives an estimation of the mass concentration in picograms per cubic centimeter and an indication of the number of other fibres in air. The size distributions of the asbestos fibres can also be provided.
Interpretation of results.	<p>The reported concentration of fibres is expected to be lower than the actual concentration, especially for amphiboles, because a variable fraction of fibres may not give identifiable SAED patterns. In the case of amphiboles, an over-estimation of the mass concentration may be made because of the manner in which fibre volumes are calculated.</p> <p>The 95% confidence levels given with the results are derived from the fibre counts per grid opening and therefore provide a measure of the precision of each analysis.</p>
Principle of method.	<p>A portion of the Nuclepore filter is ashed in a low temperature oxygen plasma furnace. The ash is suspended in fibre-free water by ultrasonication. Aliquots of this suspension are transferred directly onto formvar carbon-coated TEM grids which are then examined in a transmission electron microscope at a magnification of 20,000X.</p> <p>A representative area of the grid is examined and fibres in this area are counted, sized and classified to obtain size distributions of the fibres and the average number of fibres per unit area of the grid. The average number of fibres is converted to concentration of fibres per cubic centimeter and the average fibre size is used to calculate the mass concentration in picograms per cubic centimeter.</p> <p>Amphibole fibres are identified by their SAED patterns and chrysotile fibres are identified by their tubular morphology with confirmation of some fibres as chrysotile by SAED.</p>
Time required for analysis.	24 - 30 hours for sample preparation (a batch of several samples may be prepared at one time); 3 hours for electron microscopic examination; 1 hour for calculations.

Range of application.	The range of concentrations can be determined from the lower limit of detection up to a level where the loading of total particulate material is such that fibres are not obscured. This upper limitation is dependent upon different sampling parameters such as location and duration of sampling.
Standard deviation.	Within laboratory precision - approximately 25% expressed as relative standard deviation.
Accuracy.	Unavailable.
Limit of detection.	Defined as the concentration corresponding to 1 fibre counted.
Interferences and shortcomings.	Presence of some non-asbestos fibres can result in inaccurate fibre counts. Sample preparation may cause losses of fibres or break-up of fibre bundles. Presence of particulate material may obscure fibres. Identification procedures have limitations.
Minimum volume of sample	Not applicable.
Preservation and sample container.	The sample is collected on a 20 cm x 25 cm Nuclepore filter. The filter is placed on a 20 cm x 25 cm separator sheet, both folded in half, sent to the laboratory in a glassine envelope and tested as soon as possible.
Safety considerations.	Normal safety precautions.

ASBESTIFORM FIBRES IN WATER AND AIR

Transmission Electron Microscopy

1. Introduction

The method is designed to determine the number and mass of chrysotile and amphibole asbestos fibres per liter of water or per cubic centimeter in air. In general, the method will detect fibres having lengths of approximately $0.2 \mu\text{m}$ and greater.

Water samples received by the laboratory either follow the direct sample preparation procedure (Section 5.1.) followed by electron microscopic examination or, if they contain sufficient quantities of insoluble organic material, e.g. plankton etc., they follow the ashing procedure (Section 5.2.) to direct sample preparation and electron microscopic examination.

Air samples received by the laboratory follow the ashing procedure to the drop method preparation (Section 5.3.) and electron microscopic examination.

Definitions

- 1.1. **Asbestos** - a generic term for several fibrous silicate minerals of the serpentine or amphibole groups. Examples are: chrysotile, actinolite, cummingtonite-grunerite, anthophyllite, crocidolite and tremolite.
- 1.2. **Fibre** - a particle having essentially parallel sides and a length to width ratio of 3:1 or greater. An asbestos fibre may be an individual fibril or a bundle of fibrils.
- 1.3. **Fibril** - a single fibre, which cannot be separated into smaller components without losing its fibrous properties or appearance.
- 1.4. **Aspect Ratio** - the ratio of length to width.
- 1.5. **Chrysotile** - a fibrous hydrated magnesium silicate mineral of the serpentine group, consisting of curved layers which overlap to form either scrolls or concentric cylinders, usually exhibiting a tubular appearance when viewed in the transmission electron microscope. The average fibril diameter is about 300 \AA .
- 1.6. **Amphibole** - a double chain silicate consisting of Si_4O_{11} units, laterally linked by various ions such as calcium, magnesium, iron, aluminum and sodium. Amphiboles may consist of or contain fibres formed through natural growth processes and may produce fragments that conform to the definition of a fibre as a result of crushing and milling processes.

2. Interferences and Shortcomings

Since the occurrence of asbestos is widespread, background contamination is unavoidable. However, background levels can be kept in the range of 4 fibres in 20 electron microscope grid openings (200 mesh) and lower by carrying out the determination of asbestos in a clean area, as free as possible from asbestos materials and by cleaning all laboratory ware before use. The laboratory floors, ceilings, wall partitions, benching etc. should be constructed from asbestos-free materials. Materials containing asbestos should not be taken into the laboratory. Special regard must be paid to clothing, especially footwear. It is recommended that the air supply to the laboratory be passed through a 0.3 μm absolute filter.

The presence of extraneous particulate material in the sample may obscure the fibres thus creating difficulties in counting and identifying fibres.

During the sample preparation procedure, particularly during low temperature oxygen plasma treatment, losses of fibres may occur. Break up of fibres during ultrasonic treatment might produce an increase in the number of fibres actually present.

Certain mineral fragments which conform to the definition of a fibre may be reported as asbestos, e.g. hornblende or halloysite. Chrysotile fibres are identified by morphology, subject to verification of a proportion of fibres by selected area electron diffraction. This procedure reduces the risk of identifying non-asbestos fibres as chrysotile.

3. Apparatus

3.1. Instrumentation

- 3.1.1. Transmission Electron Microscope with a magnification range of 300X to 50,000X and a binocular attachment. The microscope should be capable of carrying out selected area electron diffraction (SAED). A means should be available for determining the lengths and widths of fibres, e.g. concentric circles of known diameters on the fluorescent screen.
- 3.1.2. Low Temperature Plasma System with purified oxygen supply for use as required to remove insoluble organic material from samples. The reaction chamber should be large enough to allow the sample vial to be placed in it in an upright position.
- 3.1.3. Vacuum Evaporator with rotating sample stage for carbon coating Nuclepore filters.
- 3.1.4. Ultrasonic Bath, 50 - 55 KHz, for dispersing ash and for cleaning glass and plastic ware.
- 3.1.5. Ultrasonic Probe Assembly, 20 KHz, minimum power 80 W, to aid in producing homogeneous ash suspensions.
- 3.1.6. Vacuum System, to generate a vacuum up to 500 mm Hg for water sample filtration.

3.1.7. Analytical Balance, for weighing asbestos standards.

3.2. For Sample Preparation

Water Samples Only

3.2.1. Membrane Filters

Nuclepore, 0.1 μm pore size, 25 mm in diameter, or an equivalent filter, for filtering water samples and ash suspensions. Available from Nuclepore Corp., Pleasanton, California, and from Sargent-Welch Scientific of Canada Ltd., Weston, Ontario.

Nuclepore, 0.1 μm pore size, 47 mm in diameter, or an equivalent filter, for filtering water samples.

Millipore, type AA, 0.8 μm pore size, 25 mm in diameter, or an equivalent filter, to be used as a backing filter for filtering water samples. Available from Millipore Corp., Bedford, Massachusetts and from Millipore Ltd., Mississauga, Ontario.

Millipore, type AA, 0.8 μm pore size, 47 mm in diameter, or an equivalent filter, to be used as a backing filter for filtering water samples.

3.2.2. Filtering Apparatus

Filtering assembly, with frit support and a funnel with parallel sides, for filters 25 mm in diameter, used for filtering water samples and ash suspensions.

Filtration assembly, with filter support, for filters 47 mm in diameter, to be used to filter water samples, ash suspensions, solutions and distilled water.

Filtering Flask, for use with filtering apparatus, 1000 ml.

3.2.3. Modified Jaffe Wick Washer for use in dissolving Nuclepore filters. An assembly which has been found to be satisfactory is illustrated in Figure 1. and consists of a glass petri dish, 60 mm in diameter, with cover and a stack of approximately twenty Whatman 40 (or equivalent) filter papers, 4.25 cm in diameter.

3.2.4. Scalpel or fine scissors for cutting filter sections.

Air Samples Only

3.2.5. Desiccator, for drying the sample drop on the grid.

Water and Air Samples

3.2.6. Electron microscope grids, 200 and 400 mesh standard or location grids. Grids may be formvar-coated. Carbon-coated grids are required for determination of the camera constant and for preparation of UICC samples.

- 3.2.7. Carbon rods, spectrographically pure, for carbon-coating Nuclepore filters.
- 3.2.8. Carbon grating replica, e.g. with 2160 lines per mm, for calibrating the magnification settings of the electron microscope.
- 3.2.9. Gold wire, 100 per cent, approximately 0.2 mm in diameter, for calibrating the camera constant for SAED analysis.
- 3.2.10. Tweezers, for handling filters and microscope grids.
- 3.2.11. Parafilm, for protecting cleaned glassware from contamination.
- 3.2.12. Tape, for securing filters.
- 3.2.13. Microsyringe, 10 μ l, for application of wetting agent and sample to electron microscope grid.
- 3.2.14. Beakers, 250 ml, 500 ml and 1000 ml capacity.
- 3.2.15. Graduated cylinders, 10 ml, 50 ml and 500 ml.
- 3.2.16. Pipettes, 5 ml, 10 ml, 25 ml and 50 ml capacity.
- 3.2.17. Glass vials, with caps, for use in the ashing procedure.
- 3.2.18. Petri dishes, disposable, 60 mm in diameter, for storing and coating filters.

4. Reagents

Water Samples Only

- 4.1. Mercuric chloride solution. Dissolve 2.71 g of mercuric chloride, reagent grade, in 100 ml of distilled water and filter through a 0.1 μ m pore size Nuclepore filter. Used as a preservative for water samples when required.
- 4.2. Chloroform, spectro grade, for dissolving Nuclepore filters.

Air Samples Only

- 4.3. Photo-flo.

Water and Air Samples

- 4.4. Acid Solution, for cleaning glass and plastic ware.

Add one part of nitric acid (approximately 70%), reagent grade, to two parts of distilled water.

or

Add one part of hydrochloric acid (34 - 38%), reagent grade, to one part of distilled water.

Either acid solution may be used.

- 4.5. Detergent solution. Add 20 ml of Decon-75 detergent to 1 liter of distilled water. This solution has been found to be satisfactory for cleaning glass and plastic ware.
- 4.6. Denatured ethanol, filtered through a 0.1 μm pore size Nuclepore filter, for cleaning glass and plastic ware.
- 4.7. Distilled water, filtered through a 0.1 μm pore size Nuclepore filter.
- 4.8. UICC (Union Internationale Contre le Cancer) Standard Reference Samples of asbestos, for use as standards in characterizing asbestos: chrysotile B, crocidolite, amosite and anthophyllite.
- 4.9. Molybdenum trioxide, MoO_3 , reagent grade, for use in calibrating electron microscopic image rotations.

5. Procedure

Water Samples

5.1. Sample Preparation

Please see Flow Chart Diagram describing the steps required in analysis.

- 5.1.1. Centre a 0.1 μm pore size Nuclepore filter, 25 mm in diameter, shiny surface up, with a 0.8 μm pore size Millipore filter, 25 mm in diameter, as a backing filter, on the frit support of a clean filtration assembly.
- 5.1.2. Apply vacuum to hold the filters in position, and clamp the funnel in place.
- 5.1.3. Bring the filtering flask to atmospheric pressure.
- 5.1.4. Agitate the 1 liter plastic sample container for at least 2 minutes to produce a homogeneous suspension.
- 5.1.5. Using a pipette or graduated cylinder, transfer the entire volume of sample to be filtered (minimum volume 5 ml), into the filter funnel and then apply the vacuum. If the volume of sample to be filtered exceeds the capacity of the funnel, transfer a sufficient amount to nearly fill the funnel. Apply the vacuum and add the remainder to the funnel in such a manner that the level in the funnel during the addition is maintained above the 15 ml mark.

NOTE: Adherence to these instructions is essential in order that the particulate material in the volume of sample taken for analysis be deposited uniformly on the filter surface.

NOTE: The volume of sample to be filtered depends upon the asbestos concentration, the total particulate concentration and the filter size. The minimum volume of sample that can be filtered and give an even deposition of particulate matter including asbestos, on

the filter surface is 5 ml for a filter 25 mm in diameter. The maximum volume of sample that can be conveniently filtered is approximately 50 ml.

NOTE: While the sample filtration and preparation procedures are described for filters 25 mm in diameter, equivalent filters of 47 mm diameter, together with compatible filtration assemblies, may be used. In this case the minimum sample volume that can be uniformly filtered is 50 ml, and the maximum volume that can be conveniently taken is 500 ml.

- 5.1.6. After filtration, release the vacuum and remove the funnel.
- 5.1.7. Observe the filter for the presence of particulate material. If the sample is so high in solids content that the particulate loading on the filter is judged to be sufficient to interfere with the subsequent electron microscopic examination, prepare another filter by the same procedure, reducing the volume of sample taken for filtration. If the particulate loading is considered to be excessive after filtration of the minimum volume, filter another aliquot of the sample, following the procedure for removal of insoluble organic material, (5.2). Alternatively, dilute the sample as necessary with filtered distilled water and repeat the sample preparation procedure.
- 5.1.8. If the particulate loading on the Nuclepore filter is judged to be acceptable, transfer the filter, deposit side up, to a petri dish and secure in place by taping the filter near the margin.
- 5.1.9. Place the dish containing the filter in a vacuum evaporator and position the carbon rods so that the neck of the sharpened rod is located at a distance of about 7 cm or greater from the filter. With the filter rotating and following manufacturer's instructions, deposit a layer of carbon, approximately 300 Å in thickness, on the filter. Evaporate the carbon in short bursts with a pause between each burst to avoid overheating of the filter.
- 5.1.10. Remove the dish containing the filter from the vacuum evaporator and by means of a scalpel or fine scissors, cut three sections, each approximately 3 mm x 3 mm, from the carbon-coated filter. Avoid cutting the sections near the margin of the filter. It is convenient to leave the remainder of the filter in the petri dish in case additional sections are required for analysis.
- 5.1.11. Place three electron microscope grids in the modified Jaffe Wick Washer.
- 5.1.12. Place a filter section, with the carbon layer facing down, onto the grid. Apply a drop of chloroform, approximately 10 µl in volume, onto the filter section. Repeat this procedure for each of the remaining two grids and filter sections.
- 5.1.13. Pour chloroform carefully down the inner wall of the petri dish of the modified Jaffe Wick Washer until the level rises to the top filter of the filter stack. Replace the cover on the washer and let stand until the Nuclepore filter material is dissolved (8 to 24 hours). Ensure that the chloroform does not evaporate completely from the washer by adding chloroform as required.

- 5.1.14. Allow the prepared grids to dry at room temperature prior to storage or electron microscopic examination.

Flowchart of the Method for Determining Asbestos Fibres in Water

Pretreatment of water samples with insoluble organic material.

1. Filter through 0.1 μm pore size Nuclepore filter.
2. Ash filter in vial in low temperature oxygen plasma system.
3. Resuspend ash in 10 ml of filtered (0.1 μm) water in ultrasonic cleaner.
4. Continue processing as outlined for water samples with little or no insoluble organic material.

Processing of all water samples with little or no insoluble organic material.

1. Filter through 0.1 μm pore size Nuclepore filter.
2. Coat filter with carbon.
3. Transfer section of filter to EM grid in Jaffe Wick Washer.
4. Dissolve filter with chloroform in washer (8-24 hr).
5. TEM morphology and SAED.

5.2. Sample Preparation - Removal of Insoluble Organic Material

Insoluble organic matter, such as algae, plankton, etc. may be present in some samples in sufficient amounts to interfere with fibre counting. In these cases, the material may be removed by treatment in a low temperature oxygen plasma system, following the procedure given below.

- 5.2.1. Place a 0.1 μm pore size Nuclepore filter, or its equivalent, 25 mm or 47 mm in diameter, into the appropriate filtration apparatus.
- 5.2.2. Agitate the water sample for at least 2 minutes to obtain a homogeneous suspension.
- 5.2.3. Transfer a known volume of the sample by means of a pipette into the filter funnel and filter using vacuum.
- 5.2.4. After filtration, rinse the inside of the funnel with a small amount of filtered distilled water.
- 5.2.5. Release the vacuum and carefully transfer the filter to a glass vial.
- 5.2.6. Place the vial and contents in an upright position in the reaction chamber of a low temperature oxygen plasma system.

- 5.2.7. Reduce the contents of the vial to an ash. Use a controlled evacuation procedure as for powdered samples and set the power and oxygen flow to maintain a low temperature within the reaction chamber, e.g. less than 100° C.

Usually several samples may be treated at the same time, depending upon the capacity of the reaction chamber.

- 5.2.8. Maintain the plasma until the contents of the vial are judged to be completely reduced to an ash. Usually this requires a period of 8 to 24 hours. Turn off the plasma system, leaving the reaction chamber under vacuum. When the chamber has cooled to room temperature, release the vacuum gradually to avoid loss of ash.
- 5.2.9. Remove the vial from the chamber and pipette 10 ml of filtered distilled water into the vial. Cap the vial and place it in an ultrasonic bath for a period of 15 minutes to disperse the ash.
- 5.2.10. Examine the suspension visually and if the ash has not been entirely dispersed, remove the cap from the vial, insert the microtip of an ultrasonic probe assembly into the suspension and disperse the ash for 30 seconds at a low power setting (up to an output of approximately 0.5 W/ml of suspension).
- 5.2.11. Immediately transfer the entire volume of the suspension into a filtration assembly prepared according to 5.1.1, 5.1.2. Apply the vacuum and proceed as described in the sample preparation procedure from 5.1.6.

NOTE: Filtration of the ash suspension through a Nuclepore filter 25 mm in diameter will yield a higher fibre density on the filter surface than filtration through a filter 47 mm in diameter. If the larger filter is to be used, the ash suspension must be diluted to a minimum volume of 50 ml with filtered distilled water and redispersed in an ultrasonic bath prior to filtration.

Air Samples

5.3. Sample Preparation

- 5.3.1. Cut a 6 mm (¼ inch) strip of the folded air filter.
- 5.3.2. Carefully place the strip of filter into a precleaned vial.
- 5.3.3. Proceed as in 5.2.6. to 5.2.10. inclusive.
- 5.3.4. Before applying the samples to the grid, treat the carbon-coated electron microscope grid with 1 - 5 µl of a solution of diluted Photoflo 200 to remove the hydrophobic properties of the carbon film.
- 5.3.5. Rinse a 10 µl syringe twice with the sample suspension. Deposit a 1 - 5 µl aliquot of the suspension onto the detergent treated carbon-coated grid.
- 5.3.6. Air dry the grid under vacuum in a desiccator with the drop on the grid facing downward.

- 5.3.7. Remove the grid from the desiccator and load it into the grid cartridge of the transmission electron microscope. Carry out electron microscopic examination.

5.4. Electron Microscopic Examination for Water and Air Samples

To obtain a result that is representative of the distribution of fibres on the filter within a realistic time limit, at least 2 of the prepared grids are analyzed. For water samples, at least 4 and not more than 20 grid openings are examined for each sample and, for air samples, 25 grid openings are examined. Where possible, a minimum of approximately 50 fibres are counted, sized and classified. If the fibre density on the grids is low (less than 0.5 fibres per grid opening on the average), the fibres are counted, sized and classified in 12 grid openings for water samples and 25 grid openings for air samples. The number of grid openings examined should be evenly distributed among the grids analyzed, subject to the limitation that all fibres in each grid opening must be enumerated.

The total number of grid openings examined per sample may be increased if analytical results of better precision are required than can be achieved by following the procedure outlined.

If a fibre density greater than approximately 50 fibres per grid opening for a 200 mesh grid is found, the electron microscopic examination becomes unduly arduous. If this occurs, it is recommended that the sample be re-prepared. With an air sample, a smaller aliquot of the sample could be used. With a water sample, the volume filtered could be decreased, or additional sections could be cut from the original carbon-coated filter but mounted on 400 mesh grids in place of the 200 mesh grids. This latter alternative is not recommended if many fibres are present which are relatively long with respect to the dimensions of a 400 mesh grid opening. The sample preparation and grid examination procedures described are applicable to grids of either mesh.

If excessive fibre densities, e.g. greater than approximately 300 fibres per grid opening for a 200 mesh grid, are found, so that the fibres cannot be reliably enumerated, the grids are rejected for analysis. In this case, a water sample may be diluted prior to filtration with filtered distilled water and an air sample may be resuspended in a larger volume of filtered distilled water after ashing, to reduce the fibre concentration to an acceptable level.

- 5.4.1. Select a grid prepared for analysis and with the particulate side facing down, insert the grid into the specimen chamber of the transmission electron microscope.
- 5.4.2. Observe the grid at a magnification of about 500X to check for uniformity of particulate deposition and breakage of the carbon film. Reject the grid for analysis if the particles appear to be non-uniformly distributed or if a majority of the grid openings are cracked or broken. In water sample grids if filter material is present, it may be dissolved by further treatment of the grid in the modified Jaffe Wick Washer.
- 5.4.3. If the grid is found to be acceptable for analysis, choose a predesignated or randomly selected intact grid opening. Do not examine openings near the circumference of the grid.

- 5.4.4. Determine the lengths of two adjacent sides of the grid opening by means of the calibrated measuring device on the microscope and record the measurements.
- 5.4.5. Increase the magnification setting to 20,000X.
- 5.4.6. At this magnification, the field of view is considerably smaller than a grid opening, and therefore the grid opening should be scanned in an established pattern to allow all the fibres in the entire grid opening to be counted and also to avoid counting the same fibre twice. Start the scan by positioning the field of view at a corner of the grid opening and scan from one edge of the corner along or to the adjacent edge of the corner by means of one translation control. On completion of the scan, move the field of view perpendicularly to the first scan approximately the width of the field and again scan from edge to edge on a line parallel to the first scan. Continue viewing in this manner until the entire grid opening has been scanned.
- 5.4.7. Count the fibres, excluding those of obvious biological origin, by recording as one fibre:
- each separate fibre
 - each fibre of a group of fibres intersecting one another
 - each fibre intersecting the left-hand grid bar of the opening being examined
 - each fibre intersecting the foremost grid bar of the opening being examined.

Do not count more than once, any fibre which extends into more than one grid opening. Record any masses of fibres present, but do not count them as fibres.

- 5.4.8. Measure the lengths and widths of the fibres by means of the calibrated measuring device on the microscope and record the measurements. Do not measure fibres that intersect a grid bar. Record the fibre lengths to the nearest 0.1 μm and the widths to the nearest 0.02 μm .
- 5.4.9. Examine a fibre with the binocular attachment to determine whether or not the fibre has a tubular morphology. If the fibre has a tubular morphology, record it as such.
- 5.4.10. Examine the fibre by SAED by selecting a suitable camera length setting and aperture, centering the aperture on the image or a portion of the image of the fibre and switching the microscope to the diffraction pattern. Observe the pattern with the binocular attachment. As the quality of the pattern obtained depends upon fibre size, orientation and the degree of interference from adjacent particles, try to obtain the best possible pattern by adjusting the position of the fibre with respect to the aperture. The duration of the pattern observed may be brief and the analyst must be prepared to note its characteristics quickly.

NOTE: To improve the distinctness of SAED patterns, especially those obtained from small fibres, it is advisable to select a short camera length setting and a small diffraction aperture.

- 5.4.11. If a diffraction pattern exhibiting all of the characteristics of patterns given by UICC chrysotile is obtained, record the fibre as being chrysotile, identified by SAED. If the pattern obtained exhibits the characteristics of those given by UICC amphibole asbestos, record the fibre as being an amphibole.
- 5.4.12. Record as other fibres any fibre which gives an SAED pattern which can be characterized as not being that of chrysotile or amphibole or which gives a partial or no SAED pattern. A partial pattern is considered to be one which lacks sufficient characteristics to enable the fibre to be classified as asbestos or non-asbestos.
- 5.4.13. In the same manner, classify all the fibres remaining in the grid openings to be examined by their SAED patterns, except that fibres with a tubular appearance are examined by SAED until approximately 5% of the total number of those estimated to be present have given patterns characteristic of chrysotile. At this point, provided that no such fibre has been classified as an amphibole or an other fibre, consider all fibres with a tubular appearance to be chrysotile and do not continue SAED analysis on the remainder of these fibres.

If desired for the purposes of a particular analysis, chrysotile fibres may be identified on the basis of their SAED patterns only.
- 5.4.14. Continue to select grid openings from the grids to be examined on a random or predesignated basis. Examine the grid openings until 50 fibres, if possible, have been counted, sized and classified in the required number of openings.
- 5.4.15. In order that meaningful results be obtained from the method, the fibre counts for each sample should be evaluated statistically. A suggested statistical treatment is given in Section 7.

5.5. Background Levels

- 5.5.1. All glass and plastic ware used in the analysis must be thoroughly cleaned. Rinse the ware using distilled water. Place the ware in detergent solution and clean by means of an ultrasonic bath. Rinse the ware thoroughly with filtered distilled water and then once with filtered denatured ethanol. Cover the openings of filter funnels, beakers, etc., with Parafilm, replace lids on petri dishes and cap vials if they are not to be used immediately. As an additional aid in cleaning, an acid rinse, using a solution of nitric or hydrochloric acid may be incorporated into the washing procedure prior to the initial rinse with distilled water. Using these procedures and clean room facilities, the level of asbestos contamination (usually chrysotile) should not exceed 4 fibres in 20 grid openings of a 200 mesh transmission electron microscope grid.

- 5.5.2. For each water sample or batch of samples, prepare a blank by taking an aliquot of filtered distilled water through the method, using a filter of the type representative of that used for sample filtration. Take a 25 ml aliquot if a filter 25 mm in diameter is used and a 200 ml aliquot for a filter 47 mm in diameter.

For each air sample or batch of samples, prepare a blank by taking a clean filter, a type representative of that used for sampling, and processing it through the method as described.

If the asbestos level in any sample is found to be excessive, filter or water contamination should be considered as a possible cause and checked by examination of the corresponding blank sample.

- 5.5.3. Sample containers should be periodically checked for contamination by filling representative containers, selected from the batch to be used, with filtered distilled water, treating for approximately 15 minutes in an ultrasonic bath and taking aliquots of the contents through the method. If a container is found to be contaminated, the batch should either be rejected for use or each container to be used should be cleaned as outlined in 5.5.1. Representative containers should then again be checked for contamination.

5.6. Application of UICC Reference Samples to the Identification of Asbestos Fibres

To identify asbestos fibres on the basis of their SAED patterns, it is convenient to compare the patterns with those obtained from well characterized asbestos fibres. For this purpose, UICC standard reference samples of asbestos are recommended because they are of a high degree of purity and are extensively used for medical and analytical research.

As some degree of subjectivity may be involved in assigning a fibre to a particular class on the basis of its SAED pattern, the analyst should be familiar with the appearance of a variety of patterns given by asbestos and other fibres likely to occur in samples. Published micrographs of SAED patterns of chrysotile, amphiboles and related minerals are useful for information and comparison purposes.

To prepare a given UICC sample for microscopic examination, disperse approximately 1 mg of the sample in 200 ml of filtered distilled water in an ultrasonic bath. Dilute 5 ml of the suspension to 100 ml with filtered distilled water and redisperse, to obtain a final concentration of approximately 0.25 mg/l. Using a microsyringe, deposit an aliquot of the suspension, 1 to 5 μ l in volume, onto a carbon-coated electron microscope grid. Air-dry the suspension on the grid, insert the sample into the electron microscope and examine several fibres by means of the binocular attachment at a magnification setting of 20,000X. Obtain micrographs of SAED patterns from several of the fibres.

The following characteristics of UICC fibres and SAED patterns should be noted for reference and comparison purposes.

5.6.1. Chrysotile

- 5.6.1.1. The tubular internal structure of the fibre.

- 5.6.1.2. The spacing of the layer lines of the SAED pattern corresponding to a lattice spacing of 5.3 \AA
- 5.6.1.3. The unique streaks in the first layer lines of the diffraction patterns.
- 5.6.1.4. The triple set of double spots in the second layer lines.
- 5.6.1.5. The angle between the fibre axis and the layer lines. When corrected for the relative rotation between the bright field fibre image and its SAED pattern, the angle should be 90 degrees.
- 5.6.2. Amphiboles
 - 5.6.2.1. The arrangement of diffraction spots along the layer lines. Some streaking may occur.
 - 5.6.2.2. The spacing of the layer lines of the SAED patterns. As for chrysotile, this should correspond to a repeat of 5.3 \AA
 - 5.6.2.3. The angle between the fibre axis and the layer lines should be 90 degrees, after correction for the relative rotation between the bright field fibre image and its SAED pattern.

5.7. Preparation of Carbon Coated EM Grids

- 5.7.1. Completely fill a thoroughly cleaned dish or beaker with filtered distilled water.
- 5.7.2. Clean a microscope glass slide with tissue and then dip this slide into a 3% solution of formvar in 1,2-dichloroethylene for about 5 seconds.
- 5.7.3. With one continuous motion, remove the slide from the formvar, allow the excess formvar to drip off the slide and tilt the slide vertically on to a filter paper allowing the solution to drain.
- 5.7.4. Allow the thin film left adhering to the slide to dry under a heat lamp for 5 min.
- 5.7.5. To loosen the edges of the film, scrape the sides of the slide with a razor blade.
- 5.7.6. Lower the slide slowly into the water (water surface must be absolutely clean) and float the film off. The film should separate from the slide by surface tension.
- 5.7.7. Place individually, the desired number of grids onto the floating film with the shiny side facing up.
- 5.7.8. Carefully contact the film with a piece of filter paper, simultaneously lifting the film out of the water.
- 5.7.9. Dry the grids on the filter paper at room temperature.
- 5.7.10. Carbon coat the grids in a vacuum evaporator.

5.8. Calibration of the Transmission Electron Microscope

To size microscope grid openings and to size and classify asbestos fibres in the electron microscope, it is necessary to perform the following calibrations:

- 5.8.1. **Magnification Calibration.** The concentric circles on the fluorescent screen and the graticule of the binocular attachment should be calibrated by means of a carbon grating replica. Use of the binocular attachment on some microscopes involves tilting the fluorescent screen with consequent distortion of the image. Therefore, care must be taken to compensate for this in calibrating the graticule.
- 5.8.2. **Determination of the Camera Constant.** The constant should be determined by using an electron diffraction standard, e.g. a gold film which has been evaporated onto a carbon-coated electron microscope grid.
- 5.8.3. **Determination of the Relative Angle of Rotation between the Bright Field Image and the SAED Pattern.** The angle should be determined by using a substance, e.g. molybdenum trioxide, having a recognizable feature in a known crystallographic direction.

6. Calculation and Reporting

6.1. Presentation of Results

- 6.1.1. Report the concentration of chrysotile fibres in million fibres per liter for water samples and in fibres per cubic centimeter for air samples. Report the precision of the chrysotile concentration.
- 6.1.2. Report the concentration of amphibole fibres in million fibres per liter for water samples and in fibres per cubic centimeter for air samples. Report the precision of the amphibole concentration.
- 6.1.3. Report the concentration of other fibres, in million fibres per liter, for water samples and in fibres per cubic centimeter for air samples.
- 6.1.4. With water samples, report the results to the nearest 0.2, 1 and 5 million fibres per liter for concentrations in the range less than 1, 1 to 10, 10 to 100 million fibres per liter, respectively, and to 2 significant figures for concentrations greater than 100 million fibres per liter.
- 6.1.5. Report the estimated mass concentration of chrysotile fibres in micrograms per liter for water samples and in picograms per cubic centimeter for air samples.
- 6.1.6. Report the estimated mass concentration of amphibole fibres in micrograms per liter for water samples and in picograms per cubic centimeter for air samples.
- 6.1.7. If required, length, width, and mass distributions can be tabulated.

- 6.1.8. Report the concentration corresponding to 1 fibre observed in the grid squares analyzed in million fibres per liter for water samples and in fibres per cubic centimeter for air samples.
- 6.1.9. Include in the report the analyst's observations on the sample, e.g. the numbers of fibre masses and the presence of extraneous material.

6.2. Calculations

- 6.2.1. Determine the fibre concentration of chrysotile or amphibole asbestos from the following formula:

In Water:

$$C = \frac{\bar{X} \times Af}{Ao \times V \times 1000}$$

Where:

C = concentration in million fibres per liter

\bar{X} = average number of chrysotile or amphibole fibres per grid opening

Af = effective filtration area of the Nuclepore filter, in μm^2

Ao = average area of the grid opening examined, in μm^2

V = volume of the original water sample filter, in ml

In Air:

$$C = \frac{\bar{X} \times Ag \times Vs \times Af \times 1000}{Ao \times Val \times Aal \times Va}$$

Where:

C = concentration in fibres per cubic centimeter

\bar{X} = average number of chrysotile or amphibole fibres per grid opening

Ag = average area of the grid examined, in mm^2

Vs = resuspension volume, in ml

Af = exposed area of the filter, in cm^2

Ao = average area of the grid opening examined, in μm^2

Val = volume of the aliquot applied to the grid, in μl

Aal = area of the filter processed for analysis, in cm^2

Va = volume of air originally sampled, in m^3

- 6.2.2 Estimate the mass concentration of chrysotile from the following formula:

$$Mc = \frac{\pi \times C \times d}{n} \sum_{i=1}^n \left(Li \times \left(\frac{Wi}{2} \right)^2 \right)$$

Where:

Mc = estimated mass concentration of chrysotile in mg/l

C = chrysotile concentration in million fibres per liter

d = density of chrysotile, which is taken to be 2.5 g/cm³

n = number of chrysotile fibres measured

L = length of each chrysotile fibre, respectively, in mm

W = width of each chrysotile fibre, respectively, in mm

In Air

$$Mc = \frac{\pi \times C \times d}{n} \sum_{i=1}^n \left(Li \times \left(\frac{Wi}{2} \right)^2 \right)$$

Where:

Mc = estimated mass concentration of chrysotile in picograms per cubic centimeter

C = chrysotile concentration in fibres per cubic centimeter

d = density of chrysotile, which is taken to be 2.5 g/cm³

n = number of chrysotile fibres measured

L = length of each chrysotile fibre, respectively, in μ m

W = width of each chrysotile fibre, respectively, in μ m

- 6.2.3. Estimate the mass concentration of amphibole asbestos from the following formula:

In Water:

$$Ma = \frac{C \times d}{n} \sum_{i=1}^n (Li \times Wi^2)$$

Where:

Ma = estimated mass concentration of amphibole in μ g per liter

C = amphibole concentration in million fibres per liter

d = density of amphibole fibres, which is taken to be 3.25 g/cm³

n = number of amphibole fibres measured

L = length of each amphibole fibre, respectively, in μ m

W = width of each amphibole fibre, respectively, in μ m

In Air:

$$Ma = \frac{C \times d}{n} \sum_{i=1}^n (Li \times Wi^2)$$

Where:

Ma = estimated mass concentration of amphibole in picograms per cubic centimeter

C = amphibole concentration in fibres per cubic centimeter

d = density of amphibole fibres, which is taken to be 3.25 g/cm³

n = number of amphibole fibres measured

L = length of each amphibole fibre, respectively, in µm

W = width of each amphibole fibre, respectively, in µm

- 6.2.4. Determine the concentration corresponding to 1 fibre found during the analysis. This concentration represents an estimate of the detection limit:

In Water:

$$DL = \frac{Af}{N \times Ao \times V \times 1000}$$

Where:

DL = detection limit for chrysotile or amphibole asbestos in million fibres per liter

Af = effective filtration area of the Nuclepore filter, in µm²

N = number of grid openings examined for the sample

Ao = average area of the grid openings examined for the sample, in µm²

V = volume of the original water sample filtered, in ml

In Air:

$$DL = \frac{Ag \times Vs \times Af \times 1000}{N \times Ao \times Val \times Aal \times Va}$$

Where:

DL = limit for chrysotile or amphibole asbestos in fibres per cubic centimeter

Ag = average area of the grid examined, in mm²

Vs = resuspension volume, in ml

Af = exposed area of the filter, in cm²

N = number of grid openings examined for the sample

Ao = average area of the grid opening examined, in µm²

Val = volume of the aliquot applied to the grid, in µl

Aal = area of the filter processed for analysis, in cm²

Va = volume of air originally sampled, in m³

7. Precision and Accuracy

7.1. Suggested Statistical Evaluation of Grid Fibre Counts

- 7.1.1 Since the nature of the fibre distribution on the sample filter has not been fully determined, the fibre distribution obtained should be tested statistically against an assumed distribution and a measure of the precision of the analysis should be provided.
- 7.1.2 In view of the sample preparation procedures used, an approach is to consider the fibres to be uniformly and randomly distributed on the sample filter and grids. A suggested means for confirming this assumption is given below.
- 7.1.3 Determine, using the chi-square test, whether the total number of fibres found in individual grid openings are randomly and uniformly distributed among the openings, by the following formula:

$$X^2 = \sum_{i=1}^N \frac{(n_i - np_i)^2}{np_i}$$

Where:

X^2 = the chi-square statistic

n_i = observed total number of fibres in each grid opening, respectively

n = total number of fibres found in N grid openings

N = number of grid openings examined for the sample

p_i = the ratio of the area of each respective grid opening to the sum of the areas of the grid openings examined.

If the value for X^2 exceeds the value listed in statistical tables for the 0.001 significance level with $N - 1$ degrees of freedom, the fibres are not considered to be uniformly and randomly distributed. In this case, it is advisable to try to improve the uniformity of fibre deposition by re-preparing the sample and repeating the analysis. For samples for which X^2 exceeds the listed value, only an estimate of the range of the fibre concentration can be made. The upper and lower range values are obtained by substituting, respectively, the highest and lowest grid opening fibre counts for the \bar{X} term in the equation given in 6.2.1.

- 7.1.4. If uniformity and randomness of fibre deposition on the sample grids have been demonstrated as in 7.1.3., the 95% confidence interval about the mean fibre counts for chrysotile, amphibole and total asbestos may be determined using the following formulae:

$$7.1.4.1. \quad Sc = \left[\frac{N \sum_{i=1}^N X_i^2 - \left(\sum_{i=1}^N X_i \right)^2}{N(N-1)} \right]^{1/2}$$

Where:

Sc = standard deviation of the fibre counts for chrysotile

N = number of grid openings examined for the sample

X_i = number of chrysotile fibres in each grid opening, respectively

Determine the standard deviations of the fibre counts for amphibole asbestos and for total asbestos by substituting the corresponding values of X_i into equation (7.1.4.1).

$$7.1.4.2. \quad X_u = \bar{X} + \frac{tSc}{\sqrt{N}}$$

$$7.1.4.3. \quad X_l = \bar{X} - \frac{tSc}{\sqrt{N}}$$

Where:

X_u = upper value of 95% confidence interval for chrysotile

X_l = lower value of 95% confidence interval for chrysotile

\bar{X} = average number of chrysotile fibres per grid opening

t = value listed in t-distribution tables at the 95% confidence level for a two-tailed distribution with N-1 degrees of freedom

Sc = standard deviation of the fibre counts for chrysotile

N = number of grid openings examined for the sample.

The values of X_u and X_l can be converted to concentrations in million fibres per liter or fibres per cubic centimeter by using the formula in 6.2.1. and substituting either X_u or X_l for the term \bar{X} .

Obtain the upper and lower values of the 95% confidence intervals for amphibole asbestos and total asbestos by substituting the corresponding values of \bar{X} and Sc into equations (7.1.4.2) and (7.1.4.3).

Report the precision of the analysis, in terms of the upper and lower limits of the 95% confidence interval, for chrysotile, amphibole asbestos and total asbestos. If a lower limit is found to be negative, report the value of the limit as zero.

7.2. Accuracy

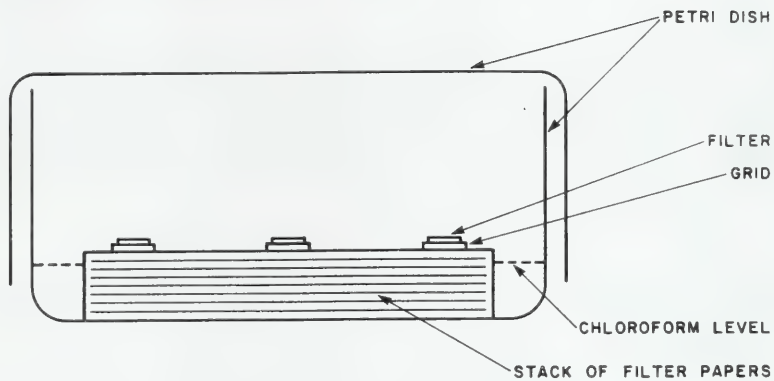
Due to the difficulty of preparing samples with a known concentration of fibres, the accuracy of these methods can not be determined directly. However, studies aimed toward the determination of the recovery of asbestos from suspension in water by calculating mass concentrations from observed

fibre dimensions have led to values ranging from 37 to 115% recovery, with a mean of 72%.

8. Bibliography

- 8.1. Flickinger, J. and J. Standridge, Identification of Fibrous Material in Two Public Water Supplies, *Environ. Sci. Technol.*, **10**, (10), 1028-1032 (October 1976).
- 8.2. Cook, P. M., G. E. Glass and J. H. Tucker, Asbestiform Amphibole Minerals: Detection and Measurement of High Concentrations in Municipal Water Supplies, *Science*, **185**, 853-855 (September, 1974).
- 8.3. Champness, P.E., G. Cliff and G.W. Lorimer, The Identification of Asbestos, *J. of Micros.*, **108**, (3), 231-249, (December 1976).
- 8.4. Interlaboratory Comparison of Selected Methods for the Determination of Asbestos Fibre Concentrations in Water, Ontario Ministry of the Environment, Laboratory Services Branch, Rexdale, Ontario, (November, 1977).
- 8.5. Anderson, C.H., and J. MacArther Long, Preliminary Interim Procedure for Fibrous Asbestos, Analytical Chemical Branch, U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, Georgia, (July, 1976).
- 8.6. An Interim Method for the Determination of Asbestos Fibre Concentrations in Water by Transmission Electron Microscopy, Ontario Ministry of the Environment, Laboratory Services Branch, Rexdale, Ontario (November, 1977).
- 8.7. An Interim Method for the Determination of Asbestiform Mineral Fibre Levels in Air and Water, Ontario Ministry of the Environment, Laboratory Services Branch, Rexdale, Ontario. (June, 1976).

(A) Washer Assembly



(B) Principle of Method

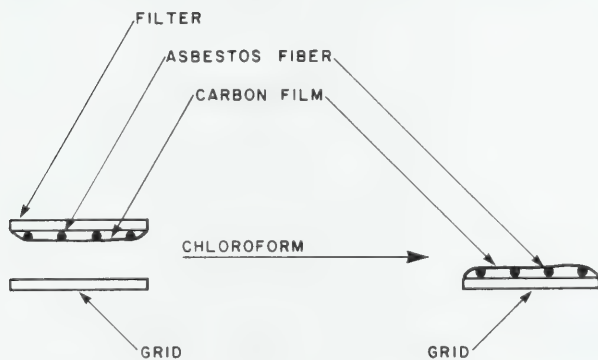


FIGURE 1 - MODIFIED JAFFE WICK WASHER AND PRINCIPLE OF METHOD.

THE DETERMINATION OF CALCIUM

Calcium is a major constituent of the geological system occurring as a carbonate, sulphate, phosphate, fluoride or silicate. Hence, calcium is also usually a major cation in natural fresh-waters, and is derived from calcareous soils and "softer" rocks which are particularly abundant throughout the southern portions of Ontario. The greatest concentrations are found in waters in the southern Grand River area, where gypsum deposits may contribute 800 mg/l or more of calcium. Normally less than 200 mg/l is found in ground waters, and less than 100 mg/l in surface waters. In hard rock areas (Precambrian Shield) there may be as little as 15 mg/l of calcium in surface waters, and occasionally less.

The calcium content of lakes may be affected, especially if shallow, by the photosynthetic activity of aquatic plants and algae. In strong sunlight, this activity may absorb so much carbon dioxide that the pH is raised to levels at which calcium carbonate is precipitated, sometimes in such quantities as to form white marl beds on the lake bottom.

The solubility of calcium carbonate is governed by the concentration of calcium bicarbonate and carbon dioxide. Any mechanism which increases the concentration of carbon dioxide increases the solubility of the carbonate.

Calcium is an essential element for all life forms. It enhances biological productivity in plants, and is required for proper bone and teeth formation in humans and other animals. Calcium is usually the major component of hardness and it is undesirable when calcium precipitation occurs in hot water tanks and distribution systems. For this reason a water softener is often installed to replace the hardness components (calcium and magnesium) by sodium.

Sample Handling and Preservation

Water, Sewage, Industrial Waste

Glass or plastic containers are acceptable. Sample containers must be filled completely so that no air remains after the containers are sealed. This will prevent the loss of dissolved carbon dioxide and the precipitation of calcium as calcium carbonate. Precipitation is not usually a problem in surface waters which are usually low in calcium, however, problems may occur in well and springwaters containing significant amounts of dissolved mineral salts. Complete filling of the sample container is not recommended during very cold weather due to the danger of freezing and possible breakage. Immediate delivery to the laboratory is recommended, especially if calcium carbonate precipitation is possible. For soil sampling see The Determination of Trace Metals by Atomic Spectroscopy.

Selection of Method

Method A, a manual compleximetric titration with EDTA is used routinely for ground water supplies where higher levels of calcium are usually encountered. Surface waters

**Preservation and
sample container.**

Polyethylene or glass containers are suitable for water samples. With samples containing large amounts of carbon dioxide the glass containers must be filled to capacity and tightly sealed to prevent loss of carbon dioxide gas. Samples should not be allowed to freeze otherwise breakage will occur.

**Safety
considerations.**

Normal laboratory safety procedures should be followed.

CALCIUM

Compleximetric Titration Method A

1. Introduction

A portion of the water sample is pipetted into a white porcelain casserole and buffered to a pH between 12 and 13. This will precipitate most of the magnesium. The sample is then titrated with 0.01M ethylenediamine tetraacetic acid (EDTA). At this pH ammonium purpurate (Murexide), used as an endpoint indicator, changes from pink to purple. At this point, all of the ionic calcium has been converted to an EDTA complex. This method is used for the analysis of drinking water.

2. Interferences and Shortcomings

Samples from industrial sources with pH values significantly less than 7.0 may require a large addition of sodium hydroxide to produce the proper pH for analysis.

Orthophosphate, if present in concentrations greater than 10 mg/l P will precipitate calcium at the pH of the test and cause low results. Alkalinity in excess of 30 mg/l calcium carbonate may cause an indistinct endpoint in hard waters.

No interference is caused by copper concentrations up to 2 mg/l, iron up to 20 mg/l, manganese up to 10 mg/l and zinc, lead, aluminum and tin up to 5 mg/l.

3. Apparatus

- 3.1. Burette, 25 ml, auto-zero pressure fill type.
- 3.2. Magnetic stirrer and 1 inch spinbars (5).
- 3.3. Porcelain casseroles, 250 ml, flat bottom (20).
- 3.4. Oxford auto pipettor.
- 3.5. Volumetric pipettes, 5, 10, 25, 50, 100 ml.
- 3.6. Volumetric flask, 1 liter.
- 3.7. Reagent bottle, 1 liter, clear, with ground glass stopper.
- 3.8. Reagent bottle, 1 liter, clear, with double bored rubber stopper.
- 3.9. Polyethylene Carboy, 20 liters.

4. Reagents

- 4.1. Ethylenediamine tetraacetic acid, -disodium salt (EDTA) reagent grade, powder.
- 4.2. Ammonium purpurate (Murexide) ($C_{10}H_4N_2O_6NH_4$) indicator, tablets.

- 4.3. Anhydrous calcium carbonate (CaCO_3) reagent grade, powder.
- 4.4. Sodium hydroxide (NaOH) reagent grade, pellets.
- 4.5. Ammonium hydroxide (NH_4OH), concentrated, reagent grade.
- 4.6. Hydrochloric acid (HCl), concentrated, reagent grade.
- 4.7. Methyl red indicator.

4.8. Standard Calcium Carbonate Solution (1000 mg/l)

Weigh 1.000 g anhydrous calcium carbonate dried for 2 hr at 105°C into a 500 ml Erlenmeyer flask. Slowly add 1:1 (v/v) hydrochloric acid until all the calcium carbonate has dissolved. Add 200 ml distilled water and boil for a few minutes to expel the carbon dioxide. Cool, add a few drops of methyl red indicator and adjust to an intermediate orange color by adding 3N ammonium hydroxide or 1:1 (v/v) hydrochloric acid as required. Transfer to a 1 liter volumetric flask, fill to the mark with carbon dioxide free distilled water and mix. Store the solution in a glass reagent bottle.

4.9. Standard EDTA Titrant 0.01M (equivalent to 1.00 mg/ml (CaCO_3))

Dissolve 74.46 g disodium ethylenediamine tetraacetic acid dihydrate in 20.0 liters of distilled, deionized water. Standardize (see Determination of Hardness).

4.10. Sodium Hydroxide Solution (1N).

Dissolve 40 g sodium hydroxide in 1 liter of distilled, deionized water.

5. Procedure

- 5.1. Select a sample aliquot volume, usually 50 ml, which will require a titrant volume ranging from a low of 3 to a maximum of 15 ml. The lower titrant volume limit of 3 ml restricts loss of precision due to the measurement of small volumes. A trial titration may be necessary to gauge the proper sample volume. Use a 100 ml aliquot if the titration requires less than 3 ml of titrant.
- 5.2. Pipette this aliquot of sample into a casserole containing a spin bar.
- 5.3. Add sufficient sodium hydroxide (normally 2 ml) to raise the pH of the sample to between 12 and 13. Samples having a low pH (i.e. below 4.0) may require additional base to bring them to the required pH. Use a pH meter to determine the base requirement.
- 5.4. Add 1 tablet of ammonium purpurate (Murexide) indicator, crushing the tablet if necessary with a clean glass rod and then rinsing the rod into the casserole.
- 5.5. Place the casserole on a magnetic stirrer and commence titration with standard 0.01M EDTA.
- 5.6. With continuous stirring, titrate the sample to the ammonium purpurate (Murexide) endpoint (pink to purple).

NOTE: The titration should be performed immediately after addition of the indicator due to its instability under alkaline conditions.

6. Calculation and Reporting

Results are calculated as mg/l Ca:

$$\begin{aligned}\text{mg/l Ca} &= M \times \frac{A}{1000} \times \frac{1000}{\text{ml sample}} \times \text{AT. WGHT EDTA} \times B \\ &= \frac{0.01 \times A \times 40080 \times B}{\text{ml sample}} \\ &= \frac{A \times B \times 400.8}{\text{ml sample}}\end{aligned}$$

Where:

A = ml titrant used

B = mg CaCO_3 equivalent to 1 ml EDTA

M = molarity of EDTA

Results are reported to 1 significant figure in the 1 - 9 mg/l Ca range, 2 significant figures in the 10 - 99 mg/l Ca range and 3 significant figures in the 100 - 999 mg/l Ca range.

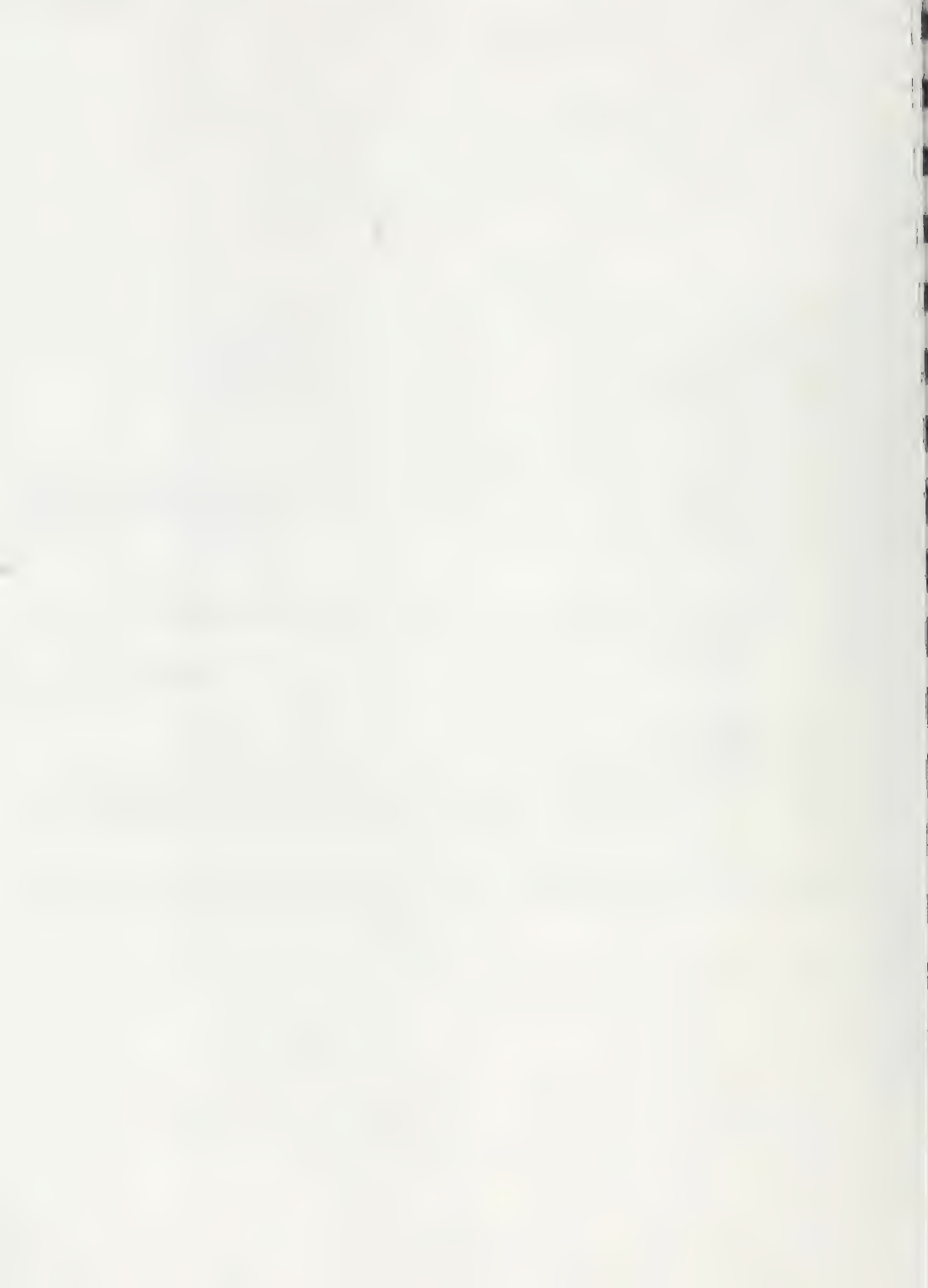
7. Precision and Accuracy

The standard deviation is ± 0.8 mg/l based on within-run duplicate samples in the 1.3 to 120 mg/l calcium range.

The standards are controlled by 2 longterm standards at 20% and 80% of the scale in such a way that the error is not greater than 2.3 mg/l.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th ed. American Public Health Association, Washington, D.C.
- 8.2. U.S. Geological Survey (1970). Study and interpretation of the chemical characteristics of water. Geological Survey Water Supply Paper No. 1473, U.S., Dept. of Interior. 363 p.



CALCIUM

Automated Atomic Absorption Spectrophotometry Method B

SUMMARY

Matrix.	This method is used on surface water and precipitation samples.
Substance determined.	Calcium as Ca.
Interpretation of results.	Results are reported as mg/l Ca.
Principle of method.	An automated atomic absorption method is used to measure the concentration of calcium ions. The sample is diluted with a releasing agent, lanthanum chloride, prior to aspiration into the burner flame.
Time required for analysis.	About 200 samples can be analyzed in one day.
Range of application.	Surface waters: a) 0.2 - 20 mg/l b) 20 - 100 mg/l Precipitation: a) 0.01 - 2.00 mg/l
Standard deviation.	Standard deviations based on within-run duplicates are as follows: surface waters (low range 0.2 - 20.0 mg/l): 0.151 for 0 - 20% of the range; 0.199 for 20 - 50% of the range and 0.178 for 50 - 100% of the range. surface waters (high range 20 - 100 mg/l): 0.99 for 20 - 50% of the range and 1.08 for 50 - 100% of the range. precipitation samples (0.06 - 2.00 mg/l range): 0.011 for 0 - 20% of the range; 0.026 for 20 - 50% of the range and 0.030 for 50 - 100% of the range.
Accuracy.	Calibration is controlled by 2 independently prepared quality control standards (QC-A and QC-B) for each range. Control is maintained in such a way that (A + B) and (A - B) do not vary by more than 3 standard deviations from the longterm mean of (A - B). These control limits are 1.3 mg/l for the high range surface waters, 0.32 mg/l for low range surface waters and 0.07 mg/l for precipitation.
Detection criteria.	0.25 for low range surface waters and 0.019 for precipitation. Insufficient data to determine detection criteria on high range surface waters.

**Interferences
and shortcomings.**

Alkali metals such as sodium, potassium, lithium and magnesium tend to cause an enhancement in absorbance, while other interferences such as aluminum, phosphate, silicate and sulphate tend to depress the calcium absorbance.

**Minimum volume
of sample.**

50 ml for surface waters and 10 ml for precipitation samples.

**Preservation and
sample container.**

Glass or plastic bottles are acceptable. With samples containing large amounts of carbon dioxide, the sample containers must be filled to capacity and tightly sealed to prevent loss of CO_2 gas. Samples should not be allowed to freeze otherwise breakage will occur.

**Safety
considerations.**

The possibility of burner flash-back or explosion is always present when using flame atomic absorption apparatus. The manufacturer's instructions for burner ignition, use, and shut-down should always be rigorously followed, and the waste trap filled with water at all times. Standard safety procedures should be employed when working with compressed gas cylinders. Caution should be exercised during the preparation of the lanthanum chloride since concentrated acid is used.

CALCIUM

Automated Atomic Absorption Spectrophotometry Method B

1. Introduction

The sample under test is automatically mixed with a releasing agent, lanthanum chloride, and aspirated as a fine mist into the air-acetylene flame of an atomic absorption spectrophotometer. Light emitted from a hollow cathode lamp at a characteristic wavelength for calcium, is directed through the flame into a monochromator and onto a detector. Calcium atoms, heated in the flame, absorb this light and the detector measures the decreased intensity of the resulting beam. The amount of light absorbed is directly proportional to the concentration of calcium in the sample and is recorded on a strip-chart recorder as a series of peaks, then compared to a calibration curve derived from the daily tested standards.

2. Interferences and Shortcomings

In an air-acetylene flame, alkali type metals such as sodium, potassium, lithium and magnesium if present in significant quantities, cause an enhancement in calcium absorbance. Other interferents such as aluminum, beryllium, chromium, iron, titanium, zinc, fluoride, sulphate, silicate and phosphate, if present in significant amounts will depress the calcium absorbance. A releasing agent such as lanthanum chloride is used to overcome the depressing effects of these interferences.

Partial clogging of the burner nebulizer and consequent reduction in aspiration may result from processing samples containing large amounts of suspended solids; pre-filtration of samples is advisable in this case.

3. Apparatus

- 3.1. Unicam SP 1900 Double Beam Atomic Absorption Spectrophotometer for surface waters and a Varian Atomic Absorption Spectrophotometer type AA275 for precipitation.
- 3.2. Double pen strip chart recorder, for surface waters. Single pen recorder for precipitation samples.
- 3.3. Automatic sample changer.
- 3.4. Gilson Minipuls II pump or equivalent.
- 3.5. Manifold as outlined in Figures 1 and 2 and 3.
- 3.6. Test tubes, 17 mm x 150 mm, for surface waters only.
- 3.7. Test tube racks for the above.

- 3.8 Plastic specimen containers, 30 ml, disposable for precipitation.
- 3.9. Air and acetylene suitable for atomic absorption analysis.
- 3.10. Voltage regulator.

NOTE: The burner head should be cleaned frequently with detergent and rinsed thoroughly with distilled water.

4. Reagents

- 4.1. Lanthanum oxide, (La_2O_3), reagent grade powder.
- 4.2. Hydrochloric acid, (HCl), concentrated, Aristar grade.
- 4.3. Calcium carbonate, (CaCO_3), reagent grade powder.
- 4.4. Ammonium Hydroxide (NH_4OH), reagent grade.
- 4.5. Methyl red indicator.

Surface Waters

4.6. Calcium Stock Solution (40,000 mg/l)

Weigh out 49.9400 g anhydrous calcium carbonate into a 500 ml Erlenmeyer flask. Slowly add 1:1 (v/v) hydrochloric acid until all the calcium carbonate has dissolved. Add 200 ml distilled, deionized water and boil for a few minutes to expel the carbon dioxide. Cool, add a few drops of methyl red indicator and adjust to the intermediate orange color by adding 3N ammonium hydroxide or 1:1 (v/v) hydrochloric acid as required. Transfer quantitatively to a 500 ml volumetric flask and dilute to the mark with distilled, deionized water.

NOTE: For magnesium, sodium and potassium stock solutions see individual methods.

4.7. Combined Intermediate Solution (0.2 - 20 mg/l range)

Pipette 10 ml calcium stock solution into a 1 liter volumetric flask. If sodium, potassium and magnesium are also to be determined transfer 5 ml sodium stock solution, 25 ml potassium stock solution and 5 ml magnesium stock solution into the flask and dilute to the mark with distilled, deionized water. This solution has the following concentrations: 400 mg/l calcium; 200 mg/l sodium; 100 mg/l potassium and 100 mg/l magnesium.

4.8. Combined Intermediate Solution (20 - 100 mg/l range)

Pipette 50 ml of each of the calcium, sodium, potassium and magnesium stock solutions into the same volumetric flask and dilute to 1 liter with distilled, deionized water. This gives the following concentrations: 2000 mg/l calcium; 2000 mg/l sodium; 200 mg/l potassium and 1000 mg/l magnesium.

4.9. Combined Calibration Standards (0.2 - 20 mg/l range)

In volumetric flasks, dilute the following aliquots of low range intermediate solution (reagent 4.7) to 1 liter with distilled, deionized water.

Aliquot	Final Concentration mg/l			
	Calcium	Sodium	Potassium	Magnesium
50 ml	20	10	5	5
40 ml	16	8	4	4
30 ml	12	6	3	3
20 ml	8	4	2	2
10 ml	4	2	1	1
5 ml	2	1	0.5	0.5

4.10. Combined Calibration Standards (20 – 100 mg/l range)

In volumetric flasks, dilute the following aliquots of high range intermediate solution (reagent 4.8) to 1 liter with distilled, deionized water.

Aliquot	Final Concentration mg/l			
	Calcium	Sodium	Potassium	Magnesium
50	100	100	10	50
40	80	80	8	40
30	60	60	6	30
20	40	40	4	20
10	20	20	2	10
5	10	10	1	5

4.11. Quality Control Stock Solution (for low range; 288 mg/l Ca)

Weight out 0.7192 g anhydrous calcium carbonate and dissolve with 1:1 (v/v) hydrochloric acid as described for reagent 4.6. Transfer quantitatively into a 1 liter volumetric flask and dilute to the mark with distilled, deionized water.

NOTE: For quality control solution use a different batch of calcium carbonate than that used for reagent 4.6. If sodium, potassium and magnesium are also to be determined prepare separate stock solutions for these as outlined in The Determination of Sodium, The Determination of Potassium and The Determination of Magnesium.

4.12. Quality Control Working Standards (0.2 – 20 mg/l range)

QC-A: Dilute 50 ml of quality control stock solution (reagent 4.11) to 1 liter in a volumetric flask with distilled, deionized water. This solution is 72% of scale.

QC-B: Dilute 20 ml of quality control stock solution (reagent 4.11) to 1 liter in a volumetric flask with distilled, deionized water. This solution is 28.8% of scale.

4.13. Quality Control Stock Solution (for high range; 1440 mg/l)

Weight out 3.5957 g anhydrous calcium carbonate and dissolve with 1:1 (v/v) hydrochloric acid as described for reagent 4.6. Transfer quantitatively to a 1 liter volumetric flask and dilute to the mark with distilled, deionized water.

NOTE: Use a different batch of calcium carbonate than the one used for reagent 4.6. If sodium, potassium and magnesium are also to be determined prepare separate stock solutions as outlined in The Determination of Sodium, The Determination of Potassium, and The Determination of Magnesium.

4.14. Quality Control Working Standards (20 - 100 mg/l range)

- QC-A: Dilute 50 ml quality control stock solution (reagent 4.13) to 1 liter in a volumetric flask with distilled, deionized water. This solution is 72% of scale.
- QC-B: Dilute 20 ml quality control stock solution (reagent 4.13) to 1 liter in a volumetric flask with distilled, deionized water. This solution is 28.8% of scale.

4.15. Lanthanum Chloride Solution (Surface Waters)

Dissolve 16.00 g lanthanum oxide in 200 ml distilled, deionized water containing 30 ml concentrated hydrochloric acid. When the salt is completely dissolved dilute to 4000 ml with distilled, deionized water and mix thoroughly. The pH of this solution should be approximately 1.7. Store solution in a plastic bottle.

Precipitation Samples

4.16. Calcium Stock Solution (4000 mg/l)

In a 500 ml volumetric flask dissolve 4.9940 g calcium carbonate (oven dried and cooled in a desiccator) as per instructions for reagent 4.6. and dilute to volume with distilled, deionized water.

NOTE: If sodium, potassium and magnesium are also to be determined prepare separate stock solutions according to the methods described in The Determination of Sodium, The Determination of Potassium and The Determination of Magnesium.

4.17. Combined Calcium-Magnesium Intermediate Solution

In a 1 liter volumetric flask, dilute 10 ml calcium stock solution and 10 ml magnesium stock solution to the mark with distilled, deionized water. This gives a calcium concentration of 40 mg/l and a magnesium concentration of 10 mg/l.

NOTE: For the measurement of sodium and potassium a combined intermediate solution is also prepared by diluting 20 ml sodium stock solution and 20 ml potassium stock solution to 1 liter with distilled, deionized water. This gives a sodium and potassium concentration of 20 mg/l. See The Determination of Sodium and The Determination of Potassium.

4.18. Combined Calcium and Magnesium Working Standards

Into volumetric flasks, pipette 5.00, 10.00, 20.00, 30.00, 40.00 and 50.00 ml aliquots of the combined intermediate solution (reagent 4.17) to 1 liter with distilled, deionized water. This gives working standards with calcium concentrations of 0.20, 0.40, 0.80, 1.20, 1.60 and 2.00 respectively and magnesium concentrations of 0.050, 0.100, 0.200, 0.300, 0.400 and 0.500 mg/l respectively.

NOTE: Similar sodium and potassium working standards are prepared by diluting aliquots of combined sodium and potassium intermediate solution to 1 liter in volumetric flasks to give a set of standards with concentrations of 0.10, 0.20, 0.40, 0.60, 0.80, 1.00 mg/l sodium respectively and 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00 mg/l potassium respectively.

4.19. Calcium Quality Control Stock Solution (4000 mg/l)

Prepare a quality control stock solution which is identical to reagent 4.16 but using a different batch of calcium carbonate.

NOTE: For sodium and potassium quality control stock solutions see The Determination of Sodium and The Determination of Potassium.

4.20. Quality Control Intermediate Solution

Pipette 10 ml calcium quality control stock solution as above (reagent 4.19) and 10 ml magnesium quality control stock solution (see The Determination of Magnesium) into a 1 liter volumetric flask to give a 10 mg/l Mg and 40 mg/l Ca solution.

NOTE: If sodium and potassium quality control intermediate solutions are also required, prepare a combined solution with 20 mg/l sodium and 20 mg/l potassium using the stock solutions for surface waters outlined in The Determination of Sodium and The Determination of Potassium.

4.21. Quality Control Working Solutions

QC-A: In a volumetric flask, dilute 30.0 ml quality control intermediate solution with distilled, deionized water to 1 liter. This solution has a calcium concentration of 1.20 mg/l and a magnesium concentration of 0.30 mg/l.

QC-B: In a volumetric flask, dilute 5.0 ml quality control intermediate solution with distilled, deionized water to 1 liter. This solution has a calcium concentration of 0.20 mg/l and a magnesium concentration of 0.050 mg/l.

NOTE: If sodium and potassium quality control working solutions are also required prepare solutions with a sodium concentration of 0.60 mg/l and potassium concentration of 0.60 mg/l for the QC-A and sodium concentration of 0.1 mg/l and a potassium concentration of 0.1 mg/l for the QC-B. See individual methods for detailed description.

NOTE: All quality control solutions should be prepared to provide test solution for at least 20 days of analysis. Prepare new QC-A's and QC-B's early and monitor their response for at least 3 days prior to adopting them.

4.22. Lanthanum Chloride Solution (Precipitation Samples)

Dissolve 8 g lanthanum oxide in 200 ml distilled, deionized water containing 20 ml concentrated hydrochloric acid. When salt is completely dissolved dilute to 1 liter with distilled, deionized water.

NOTE: Lanthanum chloride solution is proportioned with sample so that the approximate lanthanum concentration at the burner head is 680 mg/l.

5. Procedure

5.1. Surface Waters

5.1.1. Instrument Set-up

CALCIUM ANALYSES ARE PERFORMED USING AN AIR-ACETYLENE FLAME. THE FOLLOWING IS A GENERAL GUIDE TO THE INSTRUMENT. FOR MORE DETAILED INFORMATION THE OPERATOR IS REFERRED TO THE MANUFACTURER'S MANUAL.

5.1.1.1. Turn on instrument in the following sequence:

voltage stabilizer
main power switch
readout section - double pen recorder
appropriate hollow cathode lamp current

NOTE: Allow 15 minutes warm up. Ensure that there is sufficient water in the waste trap.

5.1.1.2. Set the following controls to their predetermined optimum values:

lamp current = approximately 50% of rated lamp current
slit width = 0.20 nm
wavelength = 422.7 nm
burner height = 0.5 cm
fuel flow = 700 cc/min.
oxidant flow = 4.5 l/min.

5.1.1.3. Set the digital display selector switch to the set-up mode and adjust the sensitivity control knob so that the energy pointer is approximately mid point in the blue sector of the ENERGY indicator. Observe the ENERGY meter or the digital display and re-adjust the WAVELENGTH control moving it slightly to either side of the nominal setting until the maximum energy is obtained (maximum deflection to the right on the ENERGY meter scale or largest negative value on the digital display). If the energy pointer does not remain in the blue sector, adjust the sensitivity control knob and/or the lamp current output.

NOTE: Record the optimum set-up adjustment of the digital display and sensitivity. This value is to be maintained daily.

5.1.1.4. Set the selector switch to the absorption mode. The energy pointer should remain in the blue sector.

If this is not the case it is an indication that certain parameters (lamp current, wavelength, slit width or lamp alignment etc.) may have been incorrectly set. They should be checked in sequence as necessary.

5.1.1.5. Turn on the air pressure (support) and adjust to the recommended value, (4.5 l/min.).

5.1.1.6. Turn on the acetylene (fuel) to the recommended value, (700 cc/min).

5.1.1.7. Set the FUEL ON/OFF control to the UP (ON) position. When the acetylene and air have been flowing for about 5 seconds, depress and hold the IGNITION switch, releasing it when ignition occurs at the burner head.

5.1.1.8. Aspirate the suppressant, lanthanum chloride into the flame, depress the ZERO button and adjust the baseline on the recorder.

5.1.1.9. Optimizing the Signal Output

For the working range selected, aspirate the high standard into the flame and note the absorbance reading and peak height. The absorbance should read approximately 0.500 absorbance units and the peak height should be 90% of full scale. If these conditions cannot be achieved, then carefully adjust the impact head to achieve the desired result. If the system is oversensitive, re-adjust the impact head and/or rotate the burner head accordingly. Once they are achieved, optimum conditions should be recorded. They should not change drastically from day to day.

NOTE: Lock burner chamber door.

5.1.2. Run Set-up

5.1.2.1. Using a well mixed sample, rinse a test tube a minimum of three times with sample prior to introducing an aliquot for analysis. Place test tube in rack.

Samples for analysis must be at room temperature in order to avoid possible sensitivity changes.

5.1.2.2. Complete sampler loading sequence. Each run of samples includes the following units: set of standards (STDS); distilled water blank (Bl); quality control samples (A, B); sensitivity monitoring standards (H, L); samples in groups of 10 or less (10). The sample loading sequence is as follows:

H; Bl; H; Bl; H; Bl; 6 STDS; Bl; QC-A; QC-B; Bl; H; Bl; n(10), Bl; ((10) H, Bl) where n is the number of repetitive units of samples.

5.1.3. Instrument Shut-down

5.1.3.1. Shut down the instrument in the following sequence:

- Turn off Fuel supply
- Turn off Air supply
- Shut off recorder
- Shut off lamp current control
- Shut off main power switch on AAS unit
- Shut off voltage stabilizer

NOTE: The danger of burner flash-back and/or explosion is always present while using flame atomic absorption apparatus. The manufacturer's procedures should be carefully followed for ignition, use and shut-down of the burner. Normal safety precautions must always be exercised when transporting and using compressed gas cylinders.

5.2. Precipitation Samples

5.2.1. Instrument Set-up

5.2.1.1. Turn power on.

- 5.2.1.2. Install correct lamp and select appropriate current.
- 5.2.1.3. Set the following controls to their predetermined optimum values
lamp current = 3 mA
slit width = 0.5 nm
wavelength = 422.7 nm
fuel tank pressure = 10 - 12 psi
oxidant (bench supply) pressure = 60 psi
- 5.2.1.4. Adjust the lamp angle for peak transmittance and clean burner slit and nebulizer capillary.
- NOTE:** Prior to turning fuel and oxidant and lighting flame ensure that all safety checks have been made (i.e. check that fuel and oxidant connections to the instrument are secure, that the liquid trap is full and draining correctly and that the capillary line to the nebulizer is in the liquid).
- 5.2.1.5. Turn on air and acetylene at their source to the settings given above. Turn on air at instrument to approximately 7.5 l/min and acetylene at instrument to approximately 4.5 l/min. Light flame and then turn down acetylene to about 3.5 l/min.
- 5.2.1.6. Using appropriate releasing or suppressing agent, run high standard and optimize the conditions for best absorbance (refer to instrument manual). If necessary select expansion factor for this absorbance to give a full scale reading on the recorder. Record wavelength, slit width, absorbance for high standard, and expansion factor.

5.2.2. Run Set-up

- 5.2.2.1. Fill wash water container with distilled, deionized water and start proportioning pump.
- 5.2.2.2. Put correct suppressant or releasing agent on line and attach line after the mixing coil to the nebulizer.
- 5.2.2.3. Zero the baseline.
- 5.2.2.4. Pour samples and standards in polystyrene disposable tubes after rinsing once with sample or standard before filling. Use polystyrene tubes to avoid sodium contamination from glass. Store samples in these tubes with caps. These stored samples can later be run for magnesium, sodium and potassium.
- 5.2.2.5. Each run consists of: standards (STDS) (1 - 6 for particular element); blank (distilled, deionized water); QC-A; QC-B; samples in groups of 10 (10), in-run standards (20% and 80%) (IR STDS). The run sequence is as follows:
STDS; B1; QC-A; QC-B; B1; n (10, B1, IR STDS).

5.2.3. Instrument Shut-down

- 5.2.3.1. Turn off sampler. Put suppressant or releasing agent line in distilled, deionized water and detach line from pump to nebulizer and place in distilled, deionized water, in order to rinse nebulizer and burner.
- 5.2.3.2. Turn off pump.
- 5.2.3.3. Turn off acetylene at source. When line to the instrument is drained, turn off acetylene at instrument. Repeat same procedure for air supply.

6. Calculation and Reporting

After plotting a calibration curve (absorbances of standards) convert peak heights of samples to concentration. Correct for sensitivity changes using in-run standards if necessary.

Results are reported as follows:

Surface Waters

0.2 - 20 mg/l calcium report to 2 significant figures
20 - 100 mg/l calcium report to 3 significant figures

Precipitation Samples

0.01 - 2.00 mg/l calcium report to the nearest 0.01 mg/l
Values less than 0.01 mg/l are reported as such.

7. Precision and Accuracy

Standard deviations based on within-run duplicates are as follows:

Sample Type	Range (mg/l)	S _{ld}	S _{md}	S _{hd}
surface waters	0.2 - 20.0	0.151	0.199	0.178
	20 - 100	-	0.99	1.08
precipitation	0.06 - 2.00	0.034	0.026	0.030

S_{ld} = standard deviation for 0 - 20% of the range

S_{md} = standard deviation for 20 - 50% of the range

S_{hd} = standard deviation for 50 - 100% of the range

Calibration is controlled by 2 independently prepared quality control standards (QC-A and QC-B) for each range. Control is maintained in such a way that (A + B) and (A - B) do not vary by more than 3 standard deviations from the longterm mean of (A - B). These control limits are 1.3 mg/l for high range (20 - 100 mg/l) surface waters; 0.32 mg/l for low range (0.2 - 20 mg/l) surface waters and 0.07 mg/l for precipitation samples.

8. Bibliography

- 8.1. Lindow, O. (1979). Determination of Trace Levels of Calcium, Magnesium, Sodium and Potassium by Atomic Absorption Spectrophotometry - Precipitation Samples. Ministry of the Environment, Laboratory Services Branch, Water Quality Section, Rexdale, Ontario.
- 8.2. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th ed. American Public Health Association, Washington, D.C.

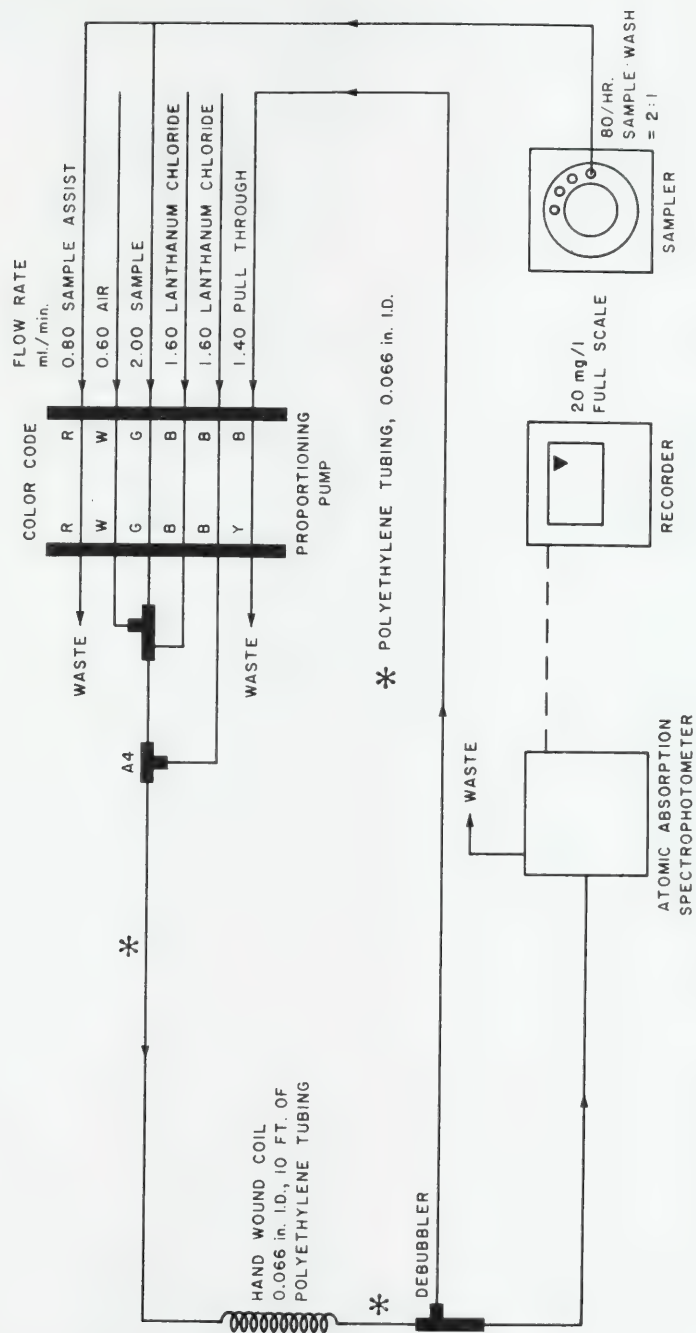


FIGURE 1 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR LOW LEVEL CALCIUM DETERMINATIONS IN SURFACE WATERS



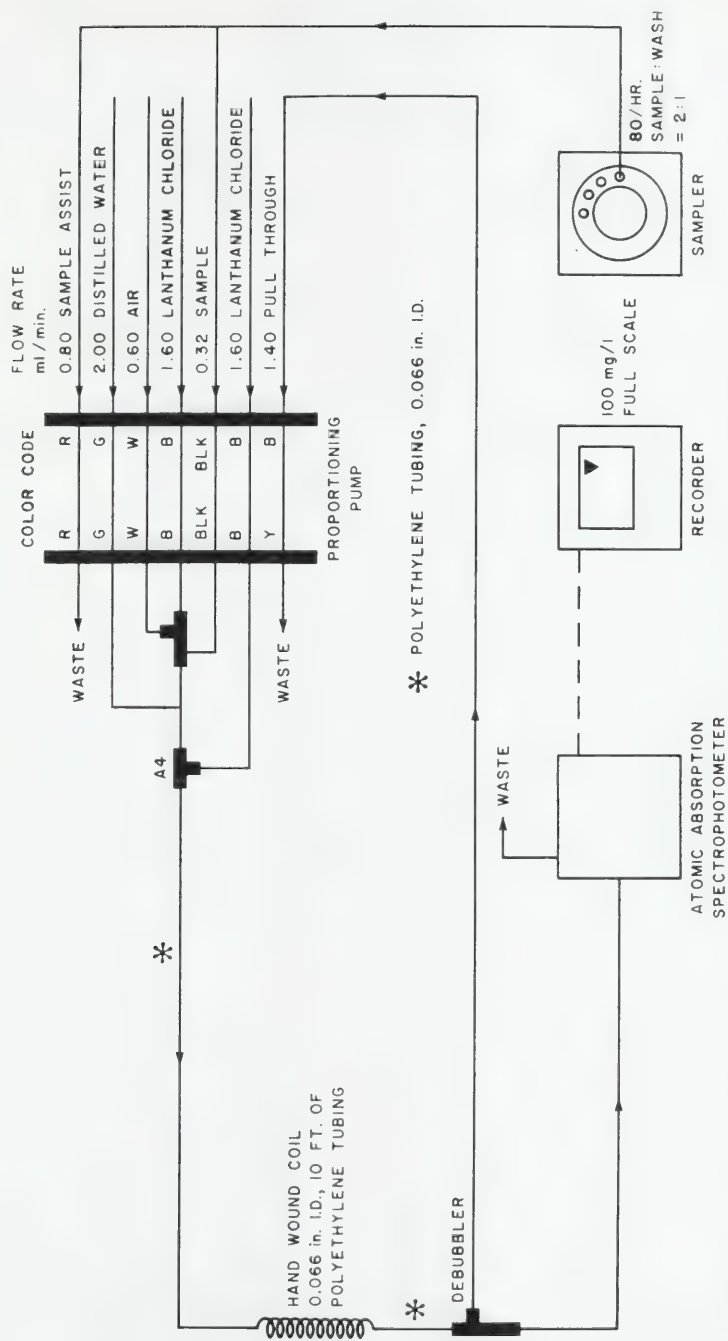


FIGURE 2 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR HIGH LEVEL CALCIUM DETERMINATIONS IN SURFACE WATERS

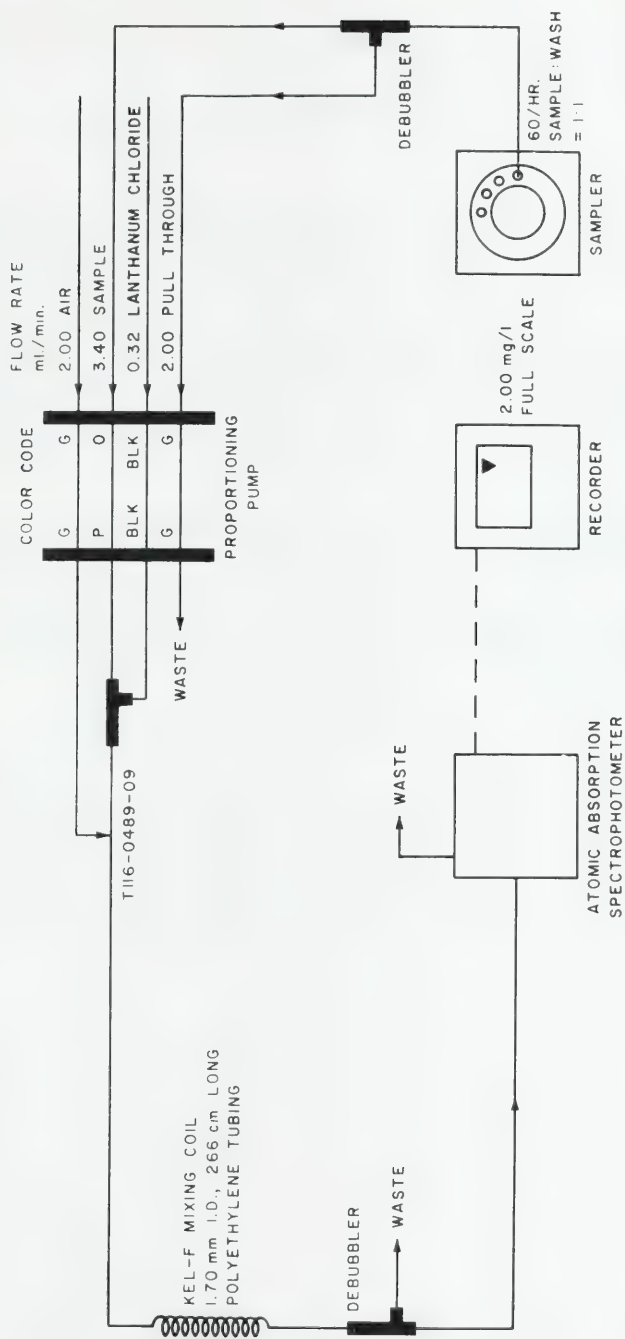


FIGURE 3 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR CALCIUM DETERMINATIONS ON PRECIPITATION SAMPLES

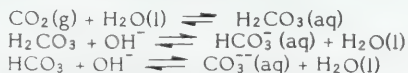
THE DETERMINATION OF CARBON

Carbon is the basic building block of all life. Inorganic and organic forms of carbon may be interconverted during the natural life cycle. Man, however, occasionally introduces alien compounds which are not beneficial in maintaining the ecological balance.

In natural waters, dissolved carbon may occur in inorganic and organic forms. The inorganic fraction can be used as an indication of the primary productivity level of lakes by providing a measure of the dissolved inorganic carbon available for uptake by algae for photosynthesis. The organic carbon content of lakes and rivers depends primarily on the products of the plants and animals supported by the water body. The degradation of large amounts of organic matter causes depletion of the dissolved oxygen concentration, and consequently, organic carbon concentrations are also measured on sewage and industrial waste.

Occasionally an empirical relationship can be established between the total organic carbon (TOC), the biological oxygen demand (BOD) and the chemical oxygen demand (COD). When this fortuitous situation is encountered, the TOC analysis provides a rapid and convenient method of estimating the BOD and COD values.

The inorganic carbon content of a freshwater sample can be correlated to total alkalinity since the latter is primarily dependent upon the carbonate-bicarbonate equilibria:



Carbon in its free or elemental form is a very noticeable indication of air pollution. Although carbon is known to cause black lung disease among miners, the effects of inhaling carbon particles present in ambient air are not known. Carbon particles possess very good adsorbing properties and therefore may be a carrier of metals or vapors. Carbon can also act as a catalyst in atmospheric reactions such as the conversion of sulphur dioxide gas to sulphuric acid aerosol. At present there are no Ontario guidelines or criteria for the amount of carbon in ambient air.

Sample Handling and Preservation

Water, Sewage and Industrial Waste (Normal range inorganic carbon)

Samples are collected in 1 litre glass or plastic storage bottles. Refrigeration and immediate transport to the laboratory for analysis are recommended.

Water (Low level range inorganic carbon)

Samples for low level carbon determinations (0-10 mg/l C) are collected in 25 x 150 mm Pyrex or Kimax tubes and sealed with screw caps having polyethylene conical liners. Care is taken to minimize sample exposure to the air. Tubes are filled to capacity and capped. Air must not be present in the container after capping. Siphoning of the sample into the container is recommended so that contact with the air is minimized. Agitation of the sample should be avoided; refrigeration and immediate transport for analysis are required.

Selection of Method

Methods A and B are used in the central laboratory for measurement of the dissolved inorganic and organic carbon contents of drinking and surface waters; these tests are also applied to the filtrate from sewage and industrial wastes. Method A, for the determination of dissolved inorganic carbon, is an automated colorimetric method involving the acidification of the sample, gas dialysis and measurement of the decrease in absorbance of a weakly buffered alkaline solution containing phenolphthalein indicator. Modifications of Method A allow low level determinations of dissolved inorganic carbon. Method B is used for dissolved organic carbon determination and involves nitrogen stripping of the inorganic carbon in the sample, oxidation by a UV digester in an acid persulphate medium, gas dialysis, and measurement of the decrease in absorbance of a weakly buffered alkaline solution containing phenolphthalein indicator.

Method C measures total and inorganic carbon by infra-red analysis and is used by the regional laboratories for carbon measurements on water, sewage and industrial wastes.

DISSOLVED INORGANIC CARBON

Automated Colorimetry Method A

SUMMARY

Matrix.	This method is used routinely on drinking water, surface water, sewage and industrial waste samples.
Substance determined	Dissolved inorganic carbon as C.
Interpretation of results.	Results are reported as mg/l carbon.
Principle of method.	The supernatant of a settled sample is segmented with carbon dioxide-free air and acidified. After gas dialysis the carbon dioxide is determined by measuring the decrease in absorbance of a weakly buffered alkaline phenolphthalein solution.
Time required for analysis.	Approximately 150 samples per day can be analyzed.
Range of application.	1) 0.3 - 40 mg/l carbon - normal range 2a) 0 - 2 mg/l carbon 2b) 0 - 10 mg/l carbon low level ranges
Standard deviation.	Normal range: 0.165 in the 0.3 - 8.0 mg/l carbon range; 0.213 in the 8.0 - 20 mg/l range; 0.310 in the 20 - 40 mg/l range. Low level range: 0.026 mg/l in the 0 - 2 mg/l; 0.061 in the 0 - 5 mg/l carbon range.
Accuracy.	Normal range: calibration is controlled by 2 independently prepared, longterm standards (QC-A and QC-B) such that (A+B) and (A-B) do not vary by more than 3 standard deviations (1.2 mg/l) from their longterm means.
Detection criteria.	Normal range: 0.27 mg/l carbon Low level range (2a): 0.043 mg/l carbon.
Interferences and shortcomings.	Chlorine and hydrogen sulphide tend to permeate the dialyzer membrane resulting in over-estimation of inorganic carbon. Sulphides interfere in low level determinations. A 1 mg/l sulphide solution gives carbon readings of 0.6 mg/l and a 10 mg/l humate solution gives a reading of 0.03 mg/l C.
Minimum volume of sample.	50 ml for the normal range.

**Preservation and
sample container.**

Normal range: samples are collected in 1 liter capacity glass or plastic storage bottles filled to capacity. Refrigeration and immediate transport are recommended.

Low level range: samples should be collected in Pyrex or Kimax tubes (25 x 150 mm) and sealed with screw caps having polyethylene conical liners. Care must be taken to minimize exposure to the air during sampling. Refrigeration and immediate transport are required.

**Safety
considerations.**

Extreme caution should be taken when preparing and using sodium hydroxide and sulphuric acid solutions.

DISSOLVED INORGANIC CARBON

Automated Colorimetry Method A

1. Introduction

Dissolved inorganic carbon plus carbon dioxide are measured by converting any carbonate or bicarbonate species to carbon dioxide. This is accomplished by acidifying the sample to pH 2.0. The sample is passed over a carbon dioxide gas permeable membrane. Carbon dioxide gas passes through the membrane and is absorbed in a weakly buffered alkaline solution containing phenolphthalein indicator. The decrease in absorbance of the phenolphthalein solution is measured by an AAI colorimeter operating in the inverse mode.

2. Interferences and Shortcomings

Any gas which permeates the dialysis membrane and affects the hydrogen ion concentration of the color solution, such as chlorine or hydrogen sulphide, will cause interferences. In the low level dissolved inorganic measurement a 1 mg/l sulphide solution was found to give a carbon reading of 0.6 mg/l.

3. Apparatus

- 3.1. Single channel (Technicon AutoAnalyzer II System or equivalent) consisting of the following modules:
 - 3.1.1. sampler
 - 3.1.2. proportioning pump
 - 3.1.3. colorimeter equipped with 550 nm filters and 5 cm flow cells
 - 3.1.4. voltage regulator
 - 3.1.5. carbon dioxide scrubber to remove carbon dioxide from air.
 - 3.1.6. gas dialyzer (6 inch path length) equipped with either Technicon or Acculab carbon dioxide permeable membranes. (2 dialyzers are required for low level dissolved inorganic carbon determinations.)
 - 3.1.7. heating bath, variable temperature (for low level determinations only).
 - 3.1.8. two-pen chart recorder
- 3.2. Pump tubing and assorted manifold glassware as indicated in Figures 1 to 3.
- 3.3. Nitrogen cylinder equipped with reducing valve (for low level determinations only).

4. Reagents

- 4.1. Sodium bicarbonate (NaHCO_3), reagent grade powder.
- 4.2. Sulphuric acid (H_2SO_4), concentrated, reagent grade.
- 4.3. Sodium hydroxide (NaOH), reagent grade pellets.
- 4.4. Disodium tetraborate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), reagent grade crystals.
- 4.5. Phenolphthalein ($\text{C}_{20}\text{H}_{14}\text{O}_4$) reagent grade powder.
- 4.6. Triton X-100, Rohm and Haas Co., wetting agent.
- 4.7. Isopropyl alcohol ($\text{C}_3\text{H}_7\text{OH}$), reagent grade liquid.
- 4.8. Methanol (CH_3OH), reagent grade liquid.
- 4.9. **Sulphuric Acid Solution (0.5N)**

Carefully add 14 ml of concentrated sulphuric acid to approximately 800 ml of distilled, deionized water. Mix thoroughly, cool, and dilute to 1 liter with distilled, deionized water.

NOTE: Eye protection must be worn during this procedure.

4.10. Triton X-100 Stock Solution

Add 50 ml of Triton X-100 wetting agent to 50 ml of isopropyl alcohol and mix thoroughly.

4.11. Triton X-100 Working Solution

Dilute 10 ml of the stock solution to 1 liter with distilled, deionized water.

4.12. Phenolphthalein Stock Solution

Dissolve 1.0 g of phenolphthalein in 100 ml methanol.

4.13. Sodium Hydroxide Solution (0.5N)

Dissolve 20 g sodium hydroxide in 900 ml of distilled, deionized water. Mix thoroughly, cool and dilute to 1 liter with distilled, deionized water.

4.14. Sodium Hydroxide Solution (6.2N)

Dissolve 250 g sodium hydroxide in 800 ml distilled, deionized water. Mix thoroughly, cool and dilute to 1 liter with distilled, deionized water.

NOTE: COMPLETE PERSONAL PROTECTION MUST BE WORN DURING THIS PROCEDURE. THIS 25% DRY WEIGHT SODIUM HYDROXIDE SOLUTION WILL CAUSE SEVERE BURNS TO UNPROTECTED AREAS.

4.15. Buffer Stock Solution

Dissolve 5.0 g sodium bicarbonate and 2.5 g disodium tetraborate decahydrate in approximately 950 ml distilled, deionized water. Using a pH meter adjust the pH to 9.45 with 0.5N sodium hydroxide solution. Dilute to 1 liter with distilled, deionized water and stopper immediately.

NOTE: This stock buffer solution must be carefully prepared or the calibration curves will not be linear.

4.16. Working Color Reagent

This reagent should be prepared at least 2 hours before use, and preferably prepared the day before. Dilute 2.0 ml of 1% phenolphthalein solution with approximately 1900 ml of distilled, deionized water in a 2 liter volumetric flask and mix. Add 50 ml of the stock buffer solution and 1.0 ml of Triton X-100 stock reagent; dilute to volume with distilled, deionized water and mix. Transfer solution to an amber glass bottle (2 liter) and either cap tightly or connect to the carbon dioxide scrubber (Figure 1a for normal range carbon, Figure 1b for low level inorganic carbon).

NOTE: LIMIT THE EXPOSURE TO AIR AS MUCH AS POSSIBLE DURING THE PREPARATION OF THIS REAGENT BECAUSE IT WILL PICK UP CARBON DIOXIDE VERY RAPIDLY.

REAGENTS 4.17 THROUGH 4.19 INCLUSIVE PERTAIN TO NORMAL RANGE INORGANIC CARBON DETERMINATIONS.

4.17. Concentrated Stock Standard Inorganic Carbon Solution (1600 mg/l carbon)

In a volumetric flask, dissolve 11.192 g sodium bicarbonate in distilled, deionized water and dilute to 1 liter. Keep refrigerated.

4.18. Working Inorganic Carbon Standards (0.3-40 mg/l carbon range)

Prepare working standards by diluting to 1 liter the following aliquots of the concentrated stock standard: 5.0, 10.0, 15.0 and 20.0 ml. This gives a set of working standards with concentrations of 8.0, 16.0, 24.0 and 32.0 mg/l carbon respectively for the normal range carbon determinations (0.3 - 40 mg/l). Keep these working standards at room temperature. If exposure to air is limited, the lifetime of the standards is 1 week.

4.19. Quality Control Solutions

Prepare in the same manner as the standards, sufficient Quality Control A, B, and Blank solutions to last for at least 50 days of analysis. These solutions must not be prepared from the same batches of chemicals as the stock standards. The concentrations of inorganic carbon (sodium bicarbonate) are 32.0 mg/l C and 16.0 mg/l C respectively for the 0.3 - 40 mg/l range. Store a supply of the day's distilled, deionized water, and designate it as QC-blank.

NOTE: To prevent the deterioration of QC-A, QC-B, and QC-blank pour these solutions into culture tubes on the day of preparation, seal and cap tubes with polyseal cone caps. Refrigerate until required.

4.20. Wash Water

Distilled, deionized water is used to obtain a baseline for the AutoAnalyzer system. Fill the bottle up prior to the day's run and connect to carbon dioxide scrubber (Figure 1a for normal range carbon, Figure 1b for low level carbon).

REAGENTS 4.21 THROUGH 4.24 INCLUSIVE PERTAIN TO THE DETERMINATION OF LOW LEVEL DISSOLVED INORGANIC CARBON.

4.21. Inorganic Carbon Concentrated Stock Solution (200 mg/l)

In a volumetric flask, dissolve 1.40 g sodium bicarbonate (dried at 110°C and cooled in a desiccator) in boiled distilled, deionized water and dilute to 1 liter. Pour above standard solution into prewashed ampoules (40 ml) and seal ampoules with an oxygen flame burner.

NOTE: STOCK SOLUTIONS, STANDARDS AND WASH WATER ARE MADE USING A 12 LITER CARBOY OF DISTILLED, DEIONIZED WATER WHICH HAS BEEN PURGED FOR 1 HOUR WITH NITROGEN GAS TO REMOVE ANY CARBON DIOXIDE.

4.22. Inorganic Carbon Intermediate Stock Solution (20.0 mg/l)

In a volumetric flask dilute 50 ml concentrated stock from the above vials to 500 ml with nitrogen gas-purged, distilled, deionized water.

4.23. Working Inorganic Carbon Standards for 2.0 mg/l Carbon (full scale)

Prepare working standards by diluting the following volumes of 20 mg/l carbon intermediate stock solution to 500 ml, in a volumetric flask with purged, distilled, deionized water: 0, 2.50, 5.00, 7.50, 10.00, 15.00, 25.00, 40.00, 50.00 ml. This gives one blank and a set of working standards with concentrations of 0.10, 0.20, 0.30, 0.40, 0.60, 1.00, 1.60, 2.00 mg/l carbon respectively. Working standards deteriorate. To prevent any air from getting into the tubes, store in Pyrex tubes with caps having polyethylene conical liners. Standards are suitable for a maximum of 2 days.

4.24. Working Inorganic Carbon Standards for 0 - 10 mg/l Carbon (full scale)

Prepare working standards by diluting the following volumes of the 200 mg/l carbon concentrated stock solution to 500 ml in a volumetric flask with purged, distilled, deionized water: 5.0, 7.50, 10.00, 12.50, 15.00, 17.50, 20.00, 25.00 ml. This gives a set of working standards with the following concentrations: 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 10.00 mg/l carbon respectively. Working standards deteriorate. To prevent any air from getting into the tubes store in Pyrex tubes with caps having polyethylene conical liners. Standards are suitable for a maximum of 2 days.

5. Procedure

5.1. Sample Preparation for Sewage and Industrial Wastes

Using a sample volume of a least 75 ml, filter sample through a Whatman 934 AH glass fibre filter. Use first 25 ml filtrate to rinse filtration unit and flask. Discard this 25 ml portion and filter remaining 50 ml sample, pouring filtrate into an identified Prince of Wales bottle. Cap immediately.

5.2. Normal Range Dissolved Inorganic Carbon Determination (0.3 - 40 mg/l carbon)

The system is assembled according to Figures 1a and 2.

- 5.2.1. Turn on colorimeter to INVERSE MODE.
- 5.2.2. Fill wash bottle to capacity with distilled, deionized water and connect to carbon dioxide scrubber (Figure 1a). The bottle is filled to minimize air pockets containing carbon dioxide which would cause baseline drift.
- 5.2.3. Pump the color reagent. This reagent should have been prepared earlier (see 4.16), and must be connected to the carbon dioxide scrubber (Figure 1a) or stoppered at all times. A half filled bottle will cause baseline drift due to absorption of carbon dioxide at the liquid air surface. The CO₂ scrubber should show a slow stream of bubbles.
- 5.2.4. Pump the remaining reagents and make sure that pulsation in the streams entering the dialyzers is minimal.
- 5.2.5. Check baselines on recorder for noise or drift. If baselines have shifted more than 10 chart units from the previous run, see Troubleshooting Section 5.5.
- 5.2.6. When baselines are acceptable the following standards are suggested: 32, 32, 32, blank, 16, 24, 32, mg/l carbon. Measure and record the QC-A, QC-B, and QC-Blank. Plot an inorganic carbon calibration curve to see if a blank correction is required. A daily calibration should be run since these standards gradually develop a blank over 1 week.
- 5.2.7. Particulates normally settle out while the sample sits in the drum. This material is not sampled provided the probe is set as high as is practical. To ensure that particulates settle out, pour the sample aliquots for at least the first 20 samples while the system is being equilibrated. If a filtered sample is required then a portion of sample must be filtered through a Whatman 934 AH filter paper.
- 5.2.8. Complete run in accordance with AutoAnalyzer procedures, i.e., sensitivity checks after every 20 samples, blanks after every 10 samples, and duplicates. At least one duplicate should be run at the beginning and at the end of the run to ensure that its inorganic carbon concentration was not affected by exposure to air.

5.3. Procedure for Low Level Dissolved Inorganic Carbon

System is assembled as in Figures 1b and 3.

- 5.3.1. Fill 12 liter carboy with distilled, deionized water and purge with nitrogen gas for 1 hour.
- 5.3.2. Turn on colorimeter to DAMP #1 and INVERSE MODE. Allow 30 minutes to warm up.
- 5.3.3. When water in carboy has been purged, siphon water to fill wash bottle to capacity. Stopper bottle and connect to carbon dioxide scrubber (Figure 1b). Open valve and allow water to fill sampler wash reservoir.
- 5.3.4. Prepare and cap working standards.
- 5.3.5. Connect previously prepared color reagent bottle to carbon dioxide scrubber.
- 5.3.6. Pump color reagent through color lines and distilled, deionized water through other reagent lines.
- 5.3.7. Ensure that carbon dioxide scrubber shows steady stream of bubbles. If not, check all fittings and tubes for leaks.
- 5.3.8. Purge the 0.5N sulphuric acid for a short period of time with nitrogen gas.
- 5.3.9. Pump reagents for about 15 minutes and make sure baseline is steady. If the baseline shifts or is noisy, or if it has shifted more than 10 chart units from the previous day, see Troubleshooting section 5.5.
- 5.3.10. Test a purged acidified blank. This should not depress the baseline by more than 2 lines.
- 5.3.11. Run a freshly purged blank and then run the working standards followed by a purged, distilled, deionized water blank. If the new blank does not depress the baseline by more than 2 lines, begin the quality control check. If the quality control standards are within the specifications, pour the samples.
- 5.3.12. Run 10 samples followed by a high for each range and a fresh blank. Continue this pattern throughout the run. If the sensitivity does not change by more than 0.03 mg/l carbon, do not make any mathematical sensitivity changes.
- 5.3.13. At least 1 duplicate should be run at the beginning and end of the sample run.
- 5.3.14. Test another purged acidified blank. If blank depresses the baseline by more than 3 lines discard the run.

5.4. Maintenance

- 5.4.1. Color reagent lines should not be washed with distilled, deionized water or acid unless a flow problem develops since a lengthy stabilizing period occurs after washing.

- 5.4.2. Ensure that working carbon standards are kept stoppered.
- 5.4.3. Replace 6.2N sodium hydroxide solutions in the carbon dioxide scrubbers monthly.

5.5. Troubleshooting

- 5.5.1. If baseline shift exceeds 10 lines from previous day:
 - 5.5.1.1. Check room temperature. If it has shifted several degrees from previous day the baseline will shift excessively but the validity of the run will not be affected. Merely reset baseline and continue. An ambient temperature rise results in a baseline drop.
 - 5.5.1.2. Check color solution and stock buffer solution. The pH and absorbance of the color reagent should be 9.29 - 9.33 and 0.68 - 0.76 respectively. The pH of the stock buffer should be 9.45. If the stock buffer solution is at fault readjust its pH and prepare fresh color reagent. If buffer solution is correct check wetting agent for bacterial growth and phenolphthalein stock for excessive evaporation. If these solutions are normal, prepare fresh color reagent.
- 5.5.2. If baseline drifts:
 - 5.5.2.1. Internal temperature changes may be occurring because colorimeter has not reached equilibrium. This is indicated by a steady drift up. Turn on colorimeter 30 minutes before run commences.
 - 5.5.2.2. One of the reagents may be cold (rare). This is indicated by a steady drift down.
- 5.5.3. If baseline is noisy:
 - 5.5.3.1. Check pump tubes and glassware for obstructions, deposits or split tubes.
 - 5.5.3.2. Check wetting agent and lines very closely, since the slow rate at which this reagent is pumped (0.10 ml/min) enhances fouling.
- 5.5.4. If baseline is noisy and drifts:
 - 5.5.4.1. Check air lines for leaks as atmospheric carbon could be entering the system. Downward drift may also occur if ammonia from the atmosphere is leaking into the system. This will affect the low level (0-10 mg/l) carbon range but will be unnoticed in the higher (0 - 40 mg/l) carbon range. A pulsating baseline accompanied by drift may suggest an airline leak causing segmentation of flow. Air leaks in the carbon dioxide scrubber lines leading to wash water or color reagent system may cause an irregular noise and baseline drift.

6. Calculation and Reporting

Using the calibration curve, convert peak heights directly to mg/l carbon. Results are reported to: 0.01 for the 2 mg/l carbon full scale range; 0.05 for the 10 mg/l full scale range; and 0.2 mg/l for the 0 - 40 mg/l carbon range.

7. Precision and Accuracy

Within run standard deviations on duplicate surface water samples are given in the following table:

Concentration Range mg/l C	S_{ld}	S_{md}	S_{hd}
0.3-40.0	0.165	0.213	0.310
0-10.0	0.026	0.061	-

S_{ld} = standard deviation for 0-20% of range
 S_{md} = standard deviation for 20-50% of range
 S_{hd} = standard deviation for 50-100% of range

Calibration of the normal range inorganic carbon system is controlled by 2 independently prepared longterm standards (QC-A and QC-B) at concentrations of 32.0 mg/l and 16.0 mg/l. Accuracy is controlled such that (A+B) and (A-B) do not vary by more than 1.2 mg/l (3 standard deviations) from their longterm means.

8. Bibliography

- 8.1. Crowther, J. and Evans, J. (1978). Dual Channel for Determination of Dissolved Organic and Inorganic Carbon. Ministry of Environment, Laboratory Services Branch, Toronto.
- 8.2. Crowther, J. and Moody, W. B. (1980). Automatic determination of inorganic carbon in surface waters. *Analytical Chimica Acta*, **120**: 305-311.
- 8.3. Goulden, P.D. (1976). Automated determination of carbon in natural waters. *Water Research* **10**: 487-490.
- 8.4. Moore, W.A., Ludzack, F.J. and Ruchhoft, C.C. (1951). Determination of oxygen-consumed values of organic wastes. *Analytical Chemistry* **23**(9): 1297-1300.

DISSOLVED ORGANIC CARBON

UV Digestion - Automated Colorimetry Method B

SUMMARY

Matrix.	This method is used routinely for drinking water, surface water, sewage, and industrial waste samples.
Substance determined.	Organic carbon as C.
Interpretation of results.	Results are reported as mg/l carbon.
Principle of method.	The supernatant of a settled sample is acidified and flushed with nitrogen to remove inorganic carbon. The sample is then oxidized via UV digestion in an acid-persulphate medium. After dialysis, the carbon dioxide content is determined by measuring the decrease in absorbance of a weakly-buffered alkaline phenolphthalein solution.
Time required for analysis.	About 150 samples can be analyzed per day.
Range of application.	0.1 to 20.0 mg/l carbon.
Standard deviation.	0.148 mg/l for the 0-20% of the range. 0.100 mg/l for the 20-50% of the range. 0.112 mg/l for 50-100% of the range.
Accuracy.	Calibration is controlled by 2 longterm standards (QC-A and QC-B) such that (A+B) and (A-B) do not vary by more than 0.6 mg/l (3 standard deviations) from their longterm means.
Detection criteria.	0.24 mg/l carbon.
Interferences and shortcomings.	Chlorine and hydrogen sulphide permeate the dialyzer membrane, affect hydrogen ion concentration of the color solution, and result in over estimations of organic carbon. Chloride levels greater than 500 mg/l also interfere. Volatile organic carbon may be lost during the inorganic carbon stripping processes.
Minimum volume of sample.	50 ml.

**Preservation and
sample container.**

Samples are collected in 1 liter glass or plastic storage bottles. Refrigeration and immediate transport for analysis are recommended.

**Safety
considerations.**

Extreme caution should be exercised when preparing and using sodium hydroxide and sulphuric acid solutions.

DISSOLVED ORGANIC CARBON

UV Digestion – Automated Colorimetry Method B

1. Introduction

The supernatant of a settled sample is introduced into an AutoAnalyzer system where it is acidified and flushed with nitrogen at 500 ml/min to remove inorganic carbon. The sample is then oxidized with a UV digester in an acid-persulphate medium. The carbon is converted to carbon dioxide and, following dialysis, is measured by determining the loss in absorbance of a weakly buffered alkaline phenolphthalein solution using an AAI colorimeter operating in the inverse mode.

2. Interferences and Shortcomings

Any gas which permeates the dialysis membrane and affects the hydrogen ion concentration of the color solution is an interferent. This includes chlorine and hydrogen sulphide. Chloride in concentrations of greater than 500 mg/l will also interfere with carbon measurements. Volatile organic carbon may be lost during the inorganic carbon stripping process.

3. Apparatus

- 3.1. Single channel Technicon AutoAnalyzer II System or equivalent consisting of the following modules:
 - 3.1.1. sampler
 - 3.1.2. proportioning pump
 - 3.1.3. colorimeter equipped with 550 nm filters and 5 cm flow cells
 - 3.1.4. voltage regulator
 - 3.1.5. UV digester (188-B097-02)
 - 3.1.6. fin cooler (B036-01) or equivalent cooling system
 - 3.1.7. carbon dioxide scrubber (T11-0415) or equivalent system to remove carbon dioxide from air (Figure 1a)
 - 3.1.8. gas dialyzer (6 inch path length) equipped with either Technicon or Acculab carbon dioxide permeable membranes.

4. Reagents

- 4.1. Potassium biphthalate ($\text{COOH.C}_6\text{H}_4.\text{COOK}$), reagent grade powder.
- 4.2. Sulphuric acid (H_2SO_4), concentrated, reagent grade.
- 4.3. Potassium persulphate ($\text{K}_2\text{S}_2\text{O}_8$), reagent grade powder.

- 4.4. Sodium hydroxide (NaOH), reagent grade pellets.
- 4.5. Disodium tetraborate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), reagent grade crystals.
- 4.6. Phenolphthalein ($\text{C}_{20}\text{H}_{14}\text{O}_4$), reagent grade powder.
- 4.7. Triton X-100, Rohm and Haas Co. wetting agent.
- 4.8. Isopropyl alcohol ($\text{C}_3\text{H}_7\text{OH}$), reagent grade liquid.
- 4.9. Methanol (CH_3OH), reagent grade liquid.
- 4.10. Reagents 4.10 to 4.16 inclusive are prepared according to Dissolved Inorganic Carbon Method A, Section 4.10 to 4.16.

4.17. Sulphuric Acid Solution (2.0N)

In a volumetric flask, carefully add 56 ml of concentrated sulphuric acid to approximately 800 ml of distilled, deionized water. Mix thoroughly, cool, and dilute to 1 liter with distilled, deionized water.

NOTE: Eye protection should be worn during this procedure.

4.18. Potassium Persulphate Solution (4% w/v)

In a volumetric flask, dissolve 40 g of potassium persulphate in 1 liter of distilled, deionized water. Heat may be required to completely solubilize the persulphate.

4.19. Concentrated Stock Standard Organic Carbon Solution (800 mg/l carbon)

Dissolve 1.700 g of potassium biphthalate in 800 ml of distilled, deionized water using a 1 liter volumetric flask; acidify with 5 ml of 1N sulphuric acid solution, and dilute to volume with distilled, deionized water. Keep refrigerated.

4.20. Working Organic Carbon Standards

In volumetric flasks dilute the following aliquots of stock standard to 1 liter with distilled, deionized water that has been acidified with 5 ml of 1N sulphuric acid: 2.50, 5.00, 10.00, 15.00, 20.00. This gives a set of working standards with 2.00, 4.00, 8.00, 12.00 and 16.00 mg/l organic carbon respectively. Keep these working standards at room temperature. Standards should be good for 1 month.

4.21. Quality Control Solutions

Prepare in the same manner as the standards, sufficient Quality Control A, B, and Blank solutions to last for at least 20 days of analysis. These solutions must not be prepared from the same batches of chemicals as the stock standards.

The concentrations of organic carbon (potassium biphthalate) for the quality control solutions are 12.0 mg/l carbon for the QC-A and 4.00 mg/l carbon for the QC-B. Store a supply of the day's distilled, deionized water, and designate it as QC-blank. All solutions should be kept refrigerated.

4.22. Wash Water

See Dissolved Inorganic Carbon Method A, Section 4.20.

5. Procedure

Refer to Section 5.1. Method A for sewage and industrial waste sample preparation. Although dissolved carbon analyses are conducted in accordance with accepted AutoAnalyzer procedure, considerable care must be taken in setting up the day's run. Almost 18 minutes elapse between sampling and the appearance of a peak on the chart recorder. The system is set up as shown in Figures 4 and 5.

- 5.1. Turn on colorimeter to INVERSE MODE and DAMP 1. Turn on UV lamp. About 30 min are required to stabilize the temperature of the colorimeters and UV lamp.
- 5.2. Fill wash water bottle (almost to overflow) with distilled, deionized water and connect to carbon dioxide scrubber (Figure 1a). The bottle is filled to minimize air pockets, as the latter contains carbon dioxide gas which would cause baseline drift. Open valve and allow water to siphon slowly into the reservoir of the sampler.
- 5.3. Pump distilled, deionized water for about 10 minutes through all reagent lines except the color reagent lines.
- 5.4. Pump the color reagent. This reagent should have been prepared earlier (see 4.16 Method A , and must be connected to the carbon dioxide scrubber or stoppered at all times. At the start of the day's run, the amber reagent bottle should be filled with color reagent (2 liter bottle) If the run is started with a half-filled bottle, the baseline will slowly drift upward due to carbon dioxide absorption at the liquid-air surface.
- 5.5. The carbon dioxide scrubber solution should now show a slow stream of air bubbles. Check the color reagent lines to ensure that pulsation is minimal, otherwise the peaks will be very noisy.
- 5.6. Pump remaining reagents and make sure that pulsation in the streams entering the dialyzers is minimal.
- 5.7. Turn nitrogen on. Adjust flow so that flowmeter reads 31 ± 3 units. (It normally fluctuates over this range.) This setting corresponds to 500 ml/min. The flow can vary between 400 and 500 ml/min. without affecting the results.
- 5.8. After pumping reagents for 15 minutes, check the baselines on the recorder. If the baselines are noisy and shift or have shifted more than 10 chart units from the previous day, the color reagent is probably at fault (see Troubleshooting, Dissolved Inorganic Carbon Method A, Section 5.5). It is extremely important to obtain steady baselines due to the 18 minute time lag between sampling and peak appearance on the chart recorder. When the baselines are acceptable, the following standards are suggested: 16, 16, 16, blank, and 2.5 mg/l. Run and record, QC-A, QC-B, and QC-Blank.

NOTE: Organic standards must be acidic to prevent bacterial growth and absorption of carbon dioxide from the air. Organic standards are stable and do not develop blanks. A calibration curve must be run when the manifold is changed or a new stock buffer solution is prepared.

- 5.9. Particulates normally settle out while the sample sits in the drum. This material will not be sampled provided the probe is set as high as is practical.

To ensure that particulates settle out, pour the sample aliquots for at least the first 20 samples while the system is being equilibrated. If a filtered sample is required, filter through a Whatman 934 AH glass fiber filter paper.

5.10. Complete run in accordance with AutoAnalyzer procedures, i.e., sensitivity checks after every 20 samples, blanks after every 10 samples, and duplicates.

5.11. Maintenance

5.11.1. Color reagent lines should not be washed with distilled, deionized water or acid unless a flow problem develops because washing necessitates a lengthy stabilizing period.

5.11.2. The quartz coil in the UV digester should be cleaned once a week by pumping a saturated solution of EDTA in sodium hydroxide through the 1.0N sulphuric acid line for approximately 15 minutes.

5.11.3. Replace 6.2N sodium hydroxide solutions in the carbon dioxide scrubbers monthly.

6. Calculation and Reporting

The peak heights are converted to the concentration units mg/l carbon by reading from the calibration curve. Results are read to the nearest 0.1 mg/l carbon and reported to the nearest 0.1 mg/l carbon.

7. Precision and Accuracy

Standard deviations of within-run duplicates are: 0.148 mg/l for 0-20% of the range; 0.100 mg/l for 20-50% of the range and 0.123 mg/l for 50-100% of the range. Calibration is controlled by 2 independently prepared quality control standards (QC-A and QC-B) with concentrations of 12.0 mg/l C and 4.0 mg/l C. Accuracy is controlled such that (A+B) and (A-B) do not vary by more than 0.6 mg/l carbon (3 standard deviations) from their longterm means.

8. Bibliography

- 8.1. Crowther, J. and Evans, J. (1978). Dual channel for determination of dissolved organic and inorganic carbon. Ministry of Environment, Laboratory Services Branch, Toronto.
- 8.2. Erhardt, M. (1969). A new method for the automatic measurement of dissolved organic carbon in sea water. Deep Sea Research 16: 393.
- 8.3. Goulden, P.D. (1976). Automated determination of carbon in natural waters. Water Research 10: 487-490.
- 8.4. Goulden, P.D. and Brooksbank, P. (1975). Automated determinations of dissolved organic carbon in lake water. Analytical Chemistry 47: 1943-1946.
- 8.5. Jirka, A. and Carter, M.J. (1976). Alternatives for measuring organic carbon in water. 7th Technicon Int'l Congress, Dec. 13-15. New York, N.Y.

- 8.6. Moore, W.A., Ludzack, F.J. and Ruchhoft, C.C. (1951). Determination of oxygen-consumed values of organic wastes. *Analytical Chemistry* 23(9): 1297-1300.

TOTAL AND INORGANIC CARBON

Infra-red Analysis Method C

SUMMARY

Matrix.	This method is used for total and inorganic carbon measurements on water, sewage and industrial effluents.
Substance determined.	Total and inorganic carbon C.
Interpretation of results.	Results are reported as mg/l carbon for total and inorganic carbon fractions. The test measures the carbon content of the dissolved fraction. Organic carbon may be obtained from the difference between total and inorganic carbon fractions and is reported in mg/l.
Principle of method.	Carbon dioxide (CO ₂) produced in the high temperature combustion tube for total carbon or in the low temperature acid decomposition tube for inorganic carbon is measured in a non-dispersive infra-red analyzer.
Time required for analysis.	Approximately 5 minutes are required for the analysis of each sample when injections for total and inorganic carbon are required. On a routine basis, approximately 60 samples can be analyzed each day.
Range of application.	0.5-100 mg/l carbon.
Standard deviation.	For total carbon, standard deviations are: 0.492 for 20-50% of range and 0.435 for 50-100% of range. For inorganic carbon, standard deviations are: 0.270 for 20-50% of range and 0.849 for 50-100% of range.
Accuracy.	Calibration is controlled by 2 independently prepared longterm standards (QC-A and QC-B, 2 sets are required, one for total carbon and one for inorganic carbon) with carbon concentrations of 70 and 30 mg/l. Control is maintained in such a way that (A+B) and (A-B) do not vary by more than 3 standard deviations (3.0 mg/l) from their longterm means.
Detection criteria.	Detection limit = 0.5 mg/l carbon.
Interferences and shortcomings.	This procedure is only applicable to homogeneous samples or samples containing small fractions of minute particules. Since the samples are injected via a micro syringe, errors in aliquoting will

occur if particulates larger than the needle bore are present in the sample. Samples containing large fractions of particulates should be filtered and the results reported as dissolved carbon, however this is usually not done unless requested. Acidified samples produce erratic carbon results.

**Minimum volume
of sample.**

25 μ l; however, samples of 100 ml are recommended.

**Preservation and
sample container.**

It is recommended that samples for carbon analysis be submitted separately in Prince of Wales bottles with polypropylene caps with tin foil liners. Preservatives must not be used. Refrigerate samples as soon as possible after collection.

**Safety
considerations.**

Normal laboratory safety precautions.

TOTAL AND INORGANIC CARBON

Infra-red Analysis Method C

1. Introduction

Inorganic and total carbon are determined by sequential injection of 25 μ l aliquots of sample into a dual channel Beckman 915 Total Organic Carbon Analyzer. The quantities of carbon dioxide generated in the acid decomposition column and in the total combustion chamber are measured by a non-dispersive infra-red analyzer. Extra dry compressed air is utilized as the carrier gas throughout. The total carbon combustion tube uses $\text{Co}(\text{NO}_3)_2$ as a catalyst and is operated at a temperature of 950°C. Phosphoric acid coated quartz chips heated to 150°C are present in the inorganic carbon combustion tube to break down inorganic carbonates.

Separate peaks are recorded for inorganic and total carbon. Total organic carbon is obtained by difference. All results are reported as mg/l carbon.

2. Interferences and Shortcomings

Since small aliquots (25 μ l) are analyzed, sampling errors occur if particulates are present. If the particles are uniformly small enough to pass unhindered through the microsyringe, this shortcoming is minimized. Filtration of the sample through Whatman 934 AH glass fiber filter paper (pore size 1 - 2 μ) that has been pre-rinsed with sample prior to taking an aliquot for analysis, permits the determination of filtered carbon.

Strongly alkaline samples reduce the useful life of the low temperature acid decomposition tube. These samples, therefore, should be neutralized prior to analysis. Samples containing significant quantities of non-combustible material will reduce the efficiency of the catalyst in the high - temperature total combustion tube and more frequent re-packing of the column will be required.

Samples containing significant quantities of organic carbon compounds that partially decompose to form carbon dioxide under the inorganic carbon test conditions will have artificially high inorganic carbon results. Finally, volatile organic compounds may be lost due to routine handling and delays in analysis. Total organic carbon determinations should be completed as soon as possible and sample exposure to the atmosphere minimized.

3. Apparatus

- 3.1. Beckman Model 915A Total Organic Carbon Analyzer.
- 3.2. Beckman Model 215 Infra-red Analyzer.
- 3.3. Beckman 10" linear Potentiometric Recorder.
- 3.4. Microsyringe - Hamilton Model CR700-50.
- 3.5. Ground glass stoppered, reagent bottles, for standards.

- 3.6. Dilution tubes.
- 3.7. Filtration assembly.
- 3.8. Reagent bottle, equipped with a reservoir suitable to prevent direct exchange of atmospheric gases with contents.

4. Reagents

- 4.1. Sodium carbonate anhydrous, (Na_2CO_3), reagent grade crystals.
- 4.2. Potassium hydrogen phthalate ($\text{COOH.C}_6\text{H}_4.\text{COOK}$), reagent grade, powder.
- 4.3. Ascarite for CO_2 scrubber.
- 4.4. Drierite for removing moisture from extra dry compressed air.
- 4.5. Compressed air cylinder, extra dry.
- 4.6. Distilled water that has been boiled to remove dissolved CO_2 , stored in a container with a reservoir containing an air trap filled with ascarite.
- 4.7. Phosphoric acid, (H_3PO_4), 85% reagent grade.
- 4.8. Beckman Inorganic Carbon Reaction Tube Recharge Kit No. 633435.
- 4.9. Beckman Catalyst Charge Kit for Ceramic Combustion Tube No. 634361.
- 4.10. **Total Carbon Stock Solution (1000 mg/l carbon).**
In a volumetric flask, dissolve 2.125 g anhydrous potassium hydrogen phthalate in carbon dioxide free, boiled, distilled, deionized water and dilute to 1 liter.
- 4.11. **Inorganic Carbon Stock Solution (1000 mg/l carbon).**
In a volumetric flask, dissolve 8.808 g dried and desiccated anhydrous sodium carbonate in carbon dioxide free, boiled, distilled, deionized water and dilute to 1 liter.
- 4.12. **Total Carbon Working Standards**
In volumetric flasks, dilute 5 and 90 mls total carbon stock solution to 1 liter with carbon dioxide free, boiled, distilled, deionized water. This gives 2 standards with carbon concentrations of 5 and 90 mg/l respectively.
- 4.13. **Inorganic Carbon Working Standards**
Prepare as for reagent 4.12. using the inorganic carbon stock solution instead of the total carbon stock solution.
- 4.14. **Total Carbon Quality Control Stock Solution**
Prepare as in reagent 4.10. using a separate batch of potassium hydrogen phthalate.
- 4.15. **Inorganic Carbon Quality Control Stock Solution**
Prepare as for reagent 4.11. using a separate batch of anhydrous sodium carbonate.

4.16. Total Carbon Quality Control Working Solutions

In volumetric flasks, dilute 70 and 30 ml total carbon quality control stock to 1 liter with carbon dioxide free, boiled, distilled, deionized water. This gives a QC-A and QC-B of 70 and 30 mg/l carbon respectively.

4.17. Inorganic Carbon Quality Control Working Solutions

Prepare as for reagent 4.16. using the inorganic carbon quality control stock solution instead of the total carbon quality control stock solution.

NOTE: These quality control solutions should be analyzed at least 3 days prior to accepting their values and sufficient solution should be prepared to permit testing for at least 1 month.

5. Procedure

REFER TO MANUFACTURER'S MANUAL FOR GENERAL OPERATING CONDITIONS FOR THE BECKMAN CARBON ANALYZER.

5.1. Start-up

- 5.1.1. Turn on column furnaces and infra-red analyzers at least 1 hour prior to instrument calibration. (Use pre-set timer or retain instrument in the ready condition with the furnaces on and a reduced gas flow (10 cc/min).
- 5.1.2. Prior to operation, ensure that the furnaces are at the proper operating temperatures. ($950 \pm 25^{\circ}\text{C}$ for total carbon, 150 ± 5 for inorganic carbon.)
- 5.1.3. Check color of the drierite in the air drying tube and ensure that at least 50% of the compound is blue before proceeding. Check color of the ascarite in the carbon dioxide scrubbing tube and ensure that at least 50% of the compound is brown before proceeding.
- 5.1.4. Turn on strip chart recorder and allow to stabilize.
- 5.1.5. Turn on compressed air supply and adjust pressure to 3.5 ± 0.5 psi. Ensure that flow rates to the total and inorganic carbon channels are 150 ± 10 ml/min and 180 ± 10 ml/min respectively.

NOTE: A small volume of carrier air pumped directly through the reference cell may help stabilize a drifting baseline during operation.

5.2. Calibration

- 5.2.1. Inject a series of water blanks into each channel to determine background.
- 5.2.2. Calibrate instrument by injecting 25 μl aliquots of the 90 mg/l working standard into each channel. Adjust control on Analyzer to read 90 mg/l.
- 5.2.3. Inject 25 μl aliquots of 5 mg/l working standard and read.
- 5.2.4. A 90% differential should occur between blanks and the 90 mg/l standard. (If blanks are running at a 3% deflection, the 90 mg/l standard is set at 93 mg/l).

5.3. Sample Analysis

- 5.3.1. Inject 25 μ l of total carbon and inorganic carbon QC-A solution. Read and record value. Similarly, read and record values for the longterm blank and QC-B solutions.
- 5.3.2. Run samples in a similar way. Samples requiring results for total organic carbon are analyzed by sequential injection for total carbon and inorganic carbon. Identify each pair of peaks on strip chart.
- 5.3.3. After every 20 samples the sensitivity is checked by re-running the 90 mg/l standard.

6. Calculation and Reporting

The calibration has a slight curvature and, therefore, a computed equation of best fit is applied. Sample peak heights are converted to carbon concentrations by application of this equation.

Results are reported to 3 significant figures where feasible.

7. Precision and Accuracy

Based on within-run duplicates, standard deviations for total carbon determinations, in the 0-100 mg/l carbon range are: 0.492 for 20-50% of the range and 0.435 for 50-100% of the range. Standard deviations for inorganic carbon determinations are 0.270 for 20-50% of the range and 0.849 for 50-100% of the range.

Accuracy is maintained by 2 independently prepared longterm standards for total carbon and 2 similar standards for inorganic carbon (QC-A and QC-B) with concentrations of 70 and 30 mg/l carbon respectively. Calibration is controlled in such a way that (A+B) and (A-B) do not vary by more than 3 standard deviations from their longterm means. These control limits are 2.5 and 3.0 mg/l carbon respectively.

8. Bibliography

- 8.1. American Public Health Association, American Waterworks Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater. 13th edition. APHA, Washington, D.C.
- 8.2. Beckman Model 915A Total Organic Carbon Analyzer, Instruction Manual (1974). Fullerton, C.A.
- 8.3. Beckman Models 215B, and 415B Infra-red Analyzers Instruction Manual (1971). Fullerton, C.A.
- 8.4. Beckman Ten-inch Laboratory Potentionmetric Recorder, Linear and Linear-log, Instruction Manual (1965). Fullerton, C.A.

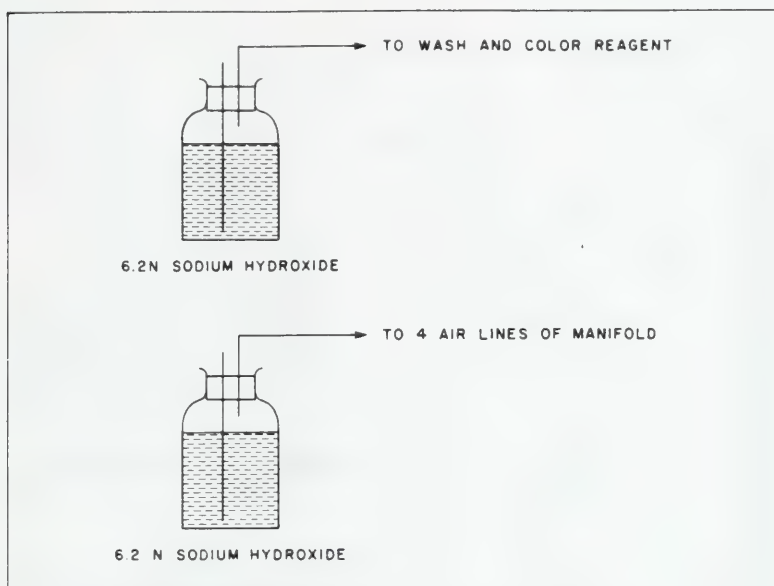


FIGURE 1a — CARBON DIOXIDE SCRUBBER FOR NORMAL RANGE INORGANIC CARBON DETERMINATION

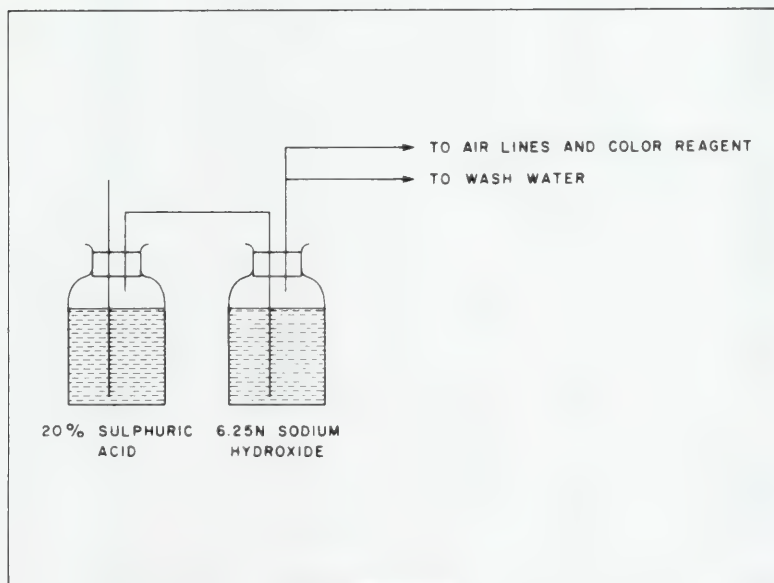


FIGURE 1b — CARBON DIOXIDE SCRUBBER FOR LOW LEVEL INORGANIC CARBON DETERMINATION

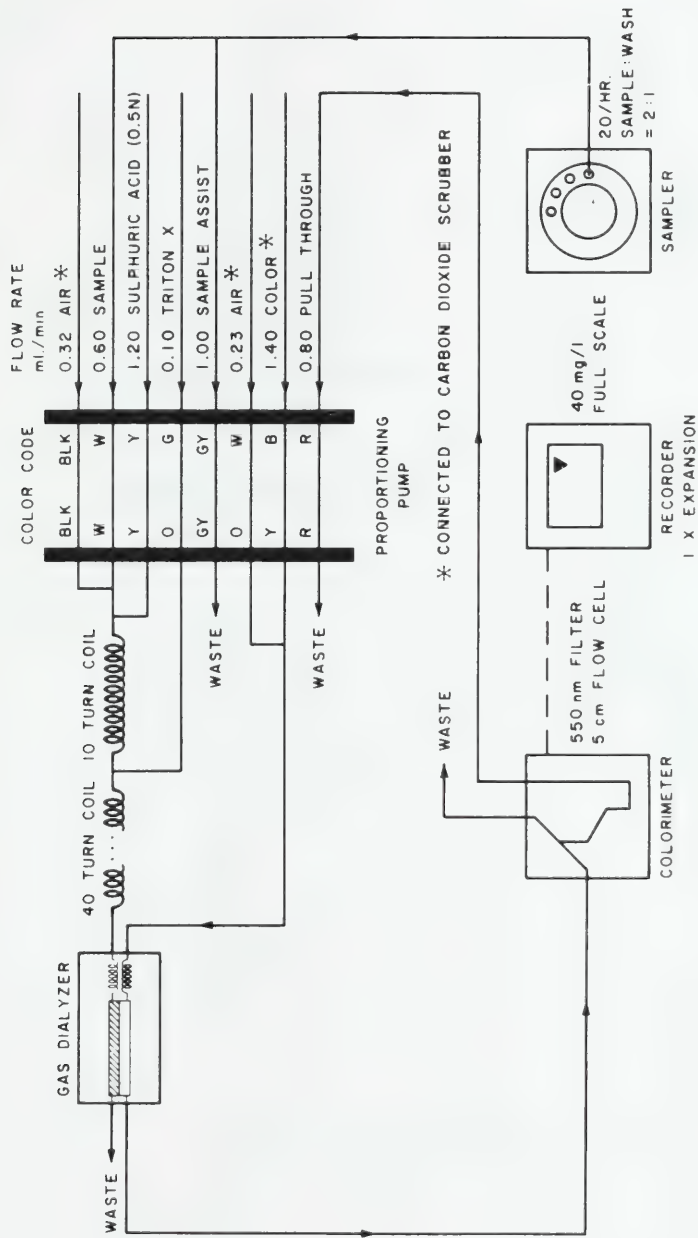


FIGURE 2 — AUTOANALYZER AAI SYSTEM FOR NORMAL RANGE DISSOLVED INORGANIC CARBON DETERMINATION

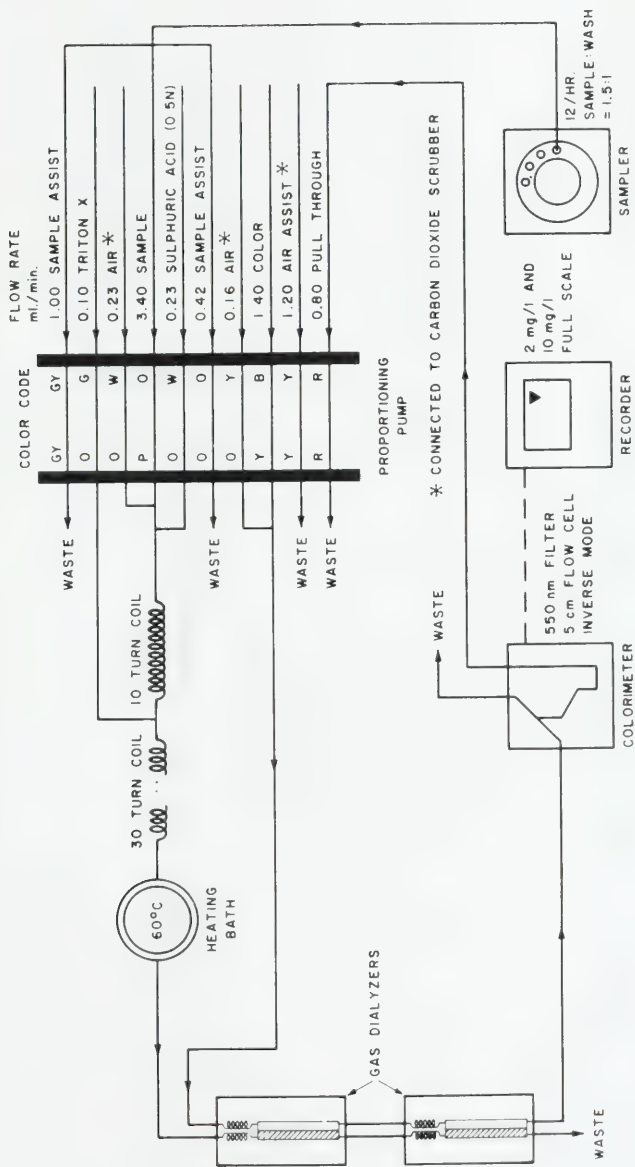


FIGURE 3 — AUTOANALYZER AAII SYSTEM FOR LOW LEVEL DISSOLVED INORGANIC CARBON DETERMINATION

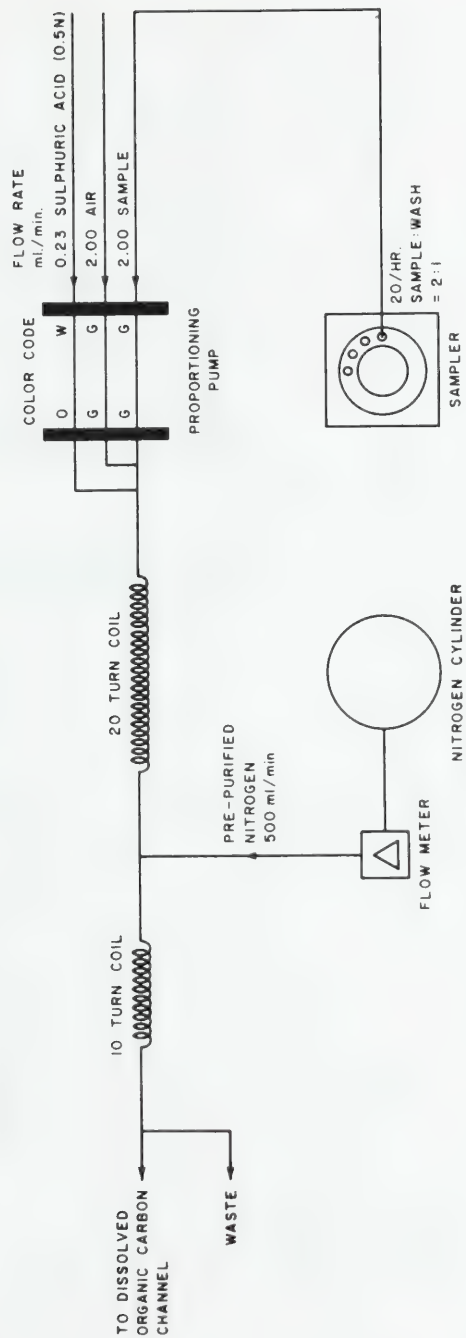


FIGURE 4 — SPARGING SYSTEM FOR DISSOLVED ORGANIC CARBON CHANNEL

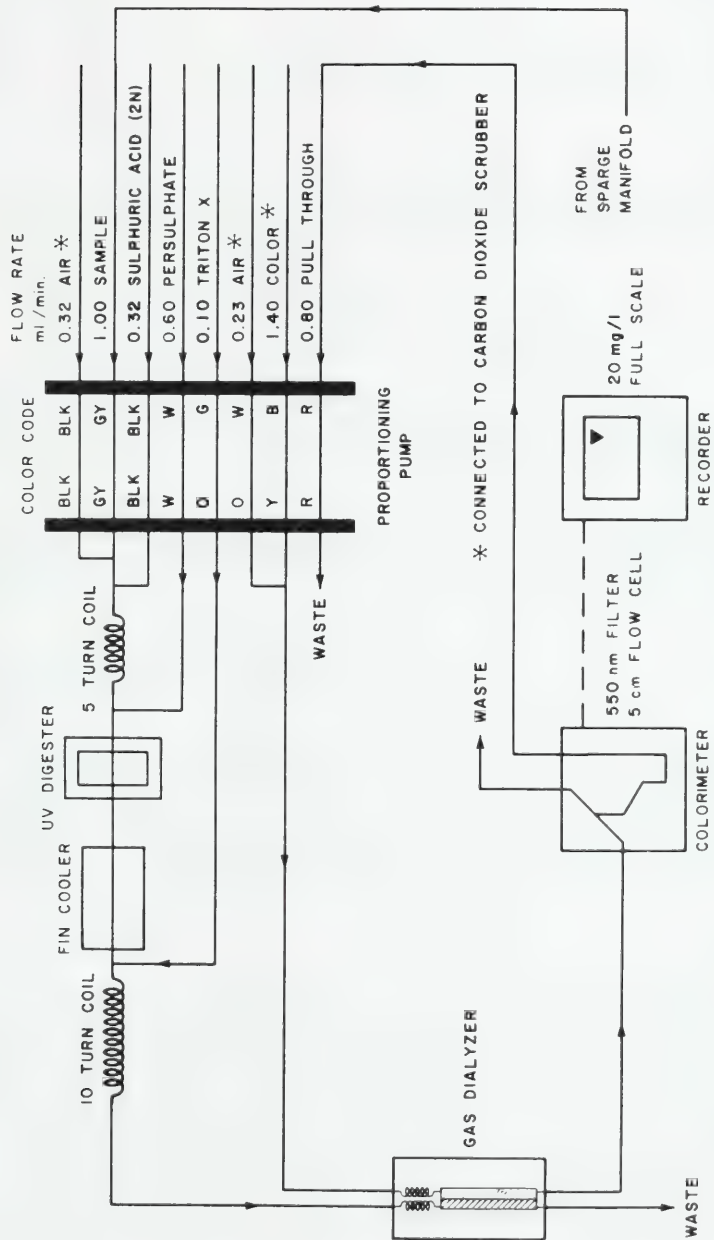
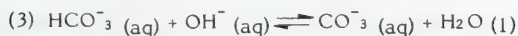
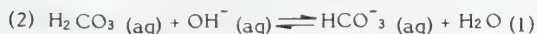


FIGURE 5 — AUTOANALYZER AAI SYSTEM FOR DISSOLVED ORGANIC CARBON DETERMINATION

THE DETERMINATION OF CARBON DIOXIDE

Carbonate equilibria in natural watersystems proceed via the equations below, where pH, the carbon dioxide partial pressure and sample alkalinity are the controlling factors.



Given the nearly constant atmospheric partial pressure of carbon dioxide (P_{CO_2} $10^{-3.5}$ atm.) and the normal alkalinity range found in surface waters, free carbon dioxide levels rarely exceed 10 mg/l. For ground waters which may experience a higher P_{CO_2} due to bacterial respiration, concentrations in excess of this level may be observed.

The carbon dioxide content of water contributes significantly to its corrosive properties; rock weathering proceeds primarily as the result of mineral dissolution and leaching by carbon dioxide saturated surface waters. The recarbonation of water during the terminal stages of water softening is a recognized treatment process. No water quality standards exist for dissolved carbon dioxide.

Sample Handling and Preservation

Water

Special precautions are required when sampling for carbon dioxide analysis. Agitation of the sample must be minimized to reduce carbon dioxide loss and samples should be carefully siphoned into the bottom of a special leakproof glass stoppered container. Copious overflow of sample prior to final capping is desirable. The presence of air bubbles in the container after capping makes carbon dioxide results unreliable if not useless. After capping, refrigerate, and analyze within 24 hours.

Selection of Method

When any three of pH, P_{CO_2} , alkalinity, and temperature (which affects carbon dioxide solubility) are known, the remaining variable may be calculated using the appropriate reaction constant expressions for the equations given above. A nomographic method for determination of carbon dioxide when pH, temperature, and alkalinity are known can be found in Standard Methods for Examination of Water and Wastewater (8.1); however, direct determination at the time of sampling is recommended. Method A, is a manual titration of aqueous carbon dioxide (carbonic acid) with carbonate free sodium hydroxide to the bicarbonate-carbonate equivalence point, defined by the phenolphthalein endpoint (pH = 8.3). This method is simple, requires minimal equipment and technician expertise, but is subject to interferences and lacks the sensitivity to resolve low concentration samples (≈ 1 mg/l as CO_2). Method B is an automated titration of aqueous carbon dioxide with carbonate free sodium hydroxide to the pH 8.3 end point. Method B is the only method currently in routine use.

CARBON DIOXIDE

Manual Titration Method A

SUMMARY

Matrix.	This method is used on ground water samples.
Substance determined.	Dissolved carbon dioxide, as CO ₂ .
Interpretation of results.	The results are reported as mg/l carbon dioxide.
Principle of method.	Free carbon dioxide is titrated against standard carbonate free sodium hydroxide to a phenolphthalein endpoint (pH = 8.3).
Time required for analysis.	Approximately 40 samples a day can be analyzed.
Range of application.	1.0 - 88 mg/l (on undiluted samples). In the unlikely event of samples with concentrations above this range, appropriate dilution with carbon dioxide free distilled water may be employed prior to titration.
Standard deviation.	0.5 mg/l.
Accuracy.	Not determined.
Detection criteria.	1.0 mg/l.
Interferences and shortcomings.	<p>Cationic and anionic species which will interfere and give high results by disturbing the carbon dioxide-carbonate equilibrium but which are generally not in sufficient concentration to be a serious problem include: aluminum, chromium, copper, and iron salts; amines, ammonia, borate, nitrite, phosphate, silicate, and sulphide. Ferrous iron should not exceed 1 mg/l. Highly colored samples will interfere with the ability to distinguish the end point.</p> <p>Mineral acid and salts of strong acids and weak bases if present will interfere.</p> <p>Negative interference will result from the presence of high dissolved solids or addition of too much indicator.</p>
Minimum volume of sample.	250 ml.

**Preservation and
sample container.**

Sample by siphoning (minimize splashing and agitation) into 250 ml glass reagent bottles fitted with ground glass stoppers. After allowing the bottle to overflow for a time, stopper so that no air bubbles remain inside. Refrigeration and immediate transport and analysis are essential.

**Safety
considerations.**

Care should be exercised when using the corrosive sodium hydroxide solution ($\approx 0.02N$) during the titration procedure.

CARBON DIOXIDE

Manual Titration Method A

1. Introduction

Free carbon dioxide is titrated with 0.02N sodium hydroxide to a phenolphthalein endpoint (colorless to pink, pH = 8.3) following reaction equations 2 and 3 noted previously.

2. Interferences and Shortcomings

Aluminum, chromium, copper, and iron salts disturb the carbon dioxide-carbonate equilibrium and lead to high results. Ferrous iron should not exceed 1 mg/l. Positive errors also result from the presence of amines, ammonia, borate, nitrite, phosphate, silicate and sulphide. Mineral acids, salts of strong acids and weak bases should be absent; hence the titrimetric carbon dioxide method is not applicable to acid mine wastes, effluent from acid regenerated cation exchangers, etc. High dissolved solids and the use of too much indicator may lead to low results.

Most surface waters and drinking water supplies contain concentrations of interfering substances which are too low to seriously affect the carbon dioxide analysis. A greater problem is the loss of carbon dioxide after sampling, during transport to the laboratory and subsequent handling. Careful sampling techniques, and subsequent sample handling, are essential.

A fading phenolphthalein end point may be observed with highly colored samples. A pH meter should be used whenever this occurs.

3. Apparatus

- 3.1. Standard glassware suitable for titration and standardization of sodium hydroxide reagent.
- 3.2. Magnetic stirrer and stirring bars.
- 3.3. pH meter with an expanded scale.

4. Reagents

- 4.1. Sodium hydroxide (NaOH), reagent grade pellets.
- 4.2. Potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$), primary standard grade.
- 4.3. Phenolphthalein indicator.
- 4.4. **Stock Sodium Hydroxide Solution (1N)**

Dissolve 40 g sodium hydroxide pellets in aqueous solution using carbon dioxide free water and dilute to 1 liter in a volumetric flask.

4.5. Working Sodium Hydroxide Titrant (0.02N)

In a volumetric flask dilute 20 ml stock sodium hydroxide solution with carbon dioxide free water (boiled and cooled distilled H₂O) and dilute to 1 liter.

4.6. Standard Phthalate Reagent (0.02N)

Dissolve 4.085 g anhydrous potassium hydrogen phthalate (dried at 110°C for 2 hours) in carbon dioxide free water and dilute to 1 liter in a volumetric flask.

4.7. Phenolphthalein Indicator

Dissolve 0.5 g phenolphthalein in 50 ml 95% ethyl or isopropyl alcohol and dilute to 100 ml with distilled water.

5. Procedure

- 5.1. Standardization: Pipet 5.0 ml of the standard potassium hydrogen phthalate solution (0.0200N) into a 200 ml Berzelius beaker. Bring the volume up to 150 ml with distilled water. With slow, continuous stirring, titrate the standard with the working sodium hydroxide titrant to a pH end point of 8.3, or, if using the phenolphthalein indicator to a pink color which persists for 30 seconds.
- 5.2. Pipet a 100 ml aliquot of sample from sample bottle into a 200 ml Berzelius beaker. Add a stirring bar.
- 5.3. Add 3 drops phenolphthalein indicator and titrate with sodium hydroxide titrant to a definite pink color which persists for 30 seconds.

or

Titrate the sample with slow continuous stirring to a pH end point of 8.3 with the standardized sodium hydroxide titrant.

NOTE: Care should be exercised to minimize any possible carbon dioxide adsorption into the sodium hydroxide titrant by keeping reagent bottles stoppered when not in immediate use.

6. Calculation and Reporting

Results are reported as mg/l CO₂

$$\text{mg/l CO}_2 = \frac{a \times n \times 44,000}{\text{ml of sample}}$$

Where:

a = ml of sodium hydroxide titrant

n = normality of sodium hydroxide titrant as determined at the beginning of each day by duplicate (minimum) standardization against 0.02 N phthalate solution.

Results are reported to 2 significant figures.

7. Precision and Accuracy

1975 data suggests that a standard deviation of 0.5 mg/l can be expected in the range 1.0-88 mg/l.

8. Bibliography

- 8.1 American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). 'Standard Methods for the Examination of Water and Wastewater. 14th ed. APHA, Washington, D. C. 293-300.
- 8.2 Garrels, R. M. and Christ C. L. (1965). Solutions, Minerals, and Equilibria. Harper & Row, New York, 77 p.
- 8.3 Ministry of the Environment. (1975). Outlines of Analytical Methods. Laboratory Services Branch, Rexdale, Ontario.

CARBON DIOXIDE
Automated Titration Method B

SUMMARY

Matrix.	This method is used routinely for carbon dioxide determinations on surface water samples.
Substance determined.	Dissolved carbon dioxide as CO ₂
Interpretation of results.	Results are reported as mg/l carbon dioxide.
Principle of method.	Free carbon dioxide is titrated automatically against standard sodium hydroxide solution to a pH endpoint (pH 8.3). The potentiometric endpoint is also determined using the titration data.
Time required for analysis.	Approximately 15 minutes is required for a single analysis although this may vary with the sample. Approximately 30 determinations may be completed per day.
Range of application.	0.01 - 10 mg/l as carbon dioxide.
Standard deviation.	0.06 mg/l in the 0 - 10 mg/l range.
Accuracy.	Not yet determined.
Detection criteria.	0.11 mg/l acidity as calcium carbonate.
Interferences and shortcomings.	The rate of titration and pH stability determine the precision and accuracy of the test. The presence of particulate matter may result in a standard deviation greater than that indicated above.
Minimum volume of sample.	100 ml; however, sample volumes in excess of 100 ml are recommended for rinsing glassware.
Preservation and sample container.	Pyrex glass or plastic sample containers are satisfactory. Ideally the bottle should be filled completely so that no bubbles remain after capping and then stored at a low temperature. No preservative is recommended and freezing should be avoided. Minimize splashing and agitation.

**Safety
considerations.**

Normal care should be exercised when using the corrosive sodium hydroxide titrant. Eye protection should be worn in making up stock and working sodium hydroxide solutions.

CARBON DIOXIDE

Automated Titration Method B

1. Introduction

The free carbon dioxide concentration of a sample is determined by automatic titration of a sample aliquot with standard sodium hydroxide ($\approx 0.01N$) to a pH of 8.3. Concentrations are calculated from the titration data. Titrant normality is determined by titration against standard 0.005N potassium hydrogen phthalate. The titrant delivery rate is controlled by the first derivative of the titration curve and by the stability of pH readings following each aliquot of titrant.

2. Interferences and Shortcomings

No significant interferences exist for the acidity determination although it is advisable to keep the sample vessel covered during the titration to minimize the effect of carbon dioxide exchange at the sample surface.

3. Apparatus

- 3.1. TRS-80 microcomputer complete with expansion interface, line printer and cassette recorder, or a PET microcomputer complete with line printer and disc drive.
- 3.2. Radiometer ABU 80 Autoburette with 2.5 ml total delivery burette assembly and BCD output.
- 3.3. Radiometer pHM 84 digital pH meter complete with BCD output.
- 3.4. In-house design interface/power supply box for interfacing the microcomputer to the Autoburette and pH meter.
- 3.5. pH electrodes.
- 3.6. Magnetic stirrer with stirring bars.
- 3.7. Berzelius beakers, 100 ml and 200 ml capacity.
- 3.8. Reagent bottles (Pyrex glass with ground glass stoppers, or nalgene with screw caps).
- 3.9. Assorted volumetric glassware (flasks and pipettes).

4. Reagents

4.1. Certified buffer solutions, pH 4.00, 6.86, 7.00 and 4.01.

4.2. Sodium hydroxide (NaOH), reagent grade pellets.

4.3. Potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$), primary standard grade.

4.4. Sulphuric acid (H_2SO_4) concentrated, reagent grade, or N/50 ampoules.

4.5. Sodium Hydroxide Stock Solution (1N)

Dissolve 40 g of sodium hydroxide pellets in 1 liter of carbon dioxide free distilled, deionized water (boiled and cooled).

4.6. Working Sodium Hydroxide Titrant ($\approx 0.01\text{N}$)

Dilute 10 ml of sodium hydroxide stock solution to 1 liter with carbon dioxide free distilled, deionized water.

4.7. Standard Potassium Hydrogen Phthalate Reagent (0.005N)

Dissolve 1.0212 g of anhydrous potassium hydrogen phthalate (previously dried at 120°C for 2 hours and cooled in a desiccator) in exactly 1 liter of carbon dioxide free distilled, deionized water (in a volumetric flask).

4.8. Quality Control Buffer 6.86

Dissolve commercially available salt according to package directions.

4.9. Quality Control Buffer 4.01

Dissolve 20.432 g of anhydrous potassium hydrogen phthalate (dried at 120°C for 2 hours and cooled in a desiccator) in 2 liters of distilled, deionized water.

4.10. Quality Control Solution A - Stock (0.005N potassium hydrogen phthalate)

Dissolve 1.0212 g of anhydrous potassium hydrogen phthalate in 1 liter of distilled, deionized water.

4.11. Quality Control Solution A - Working QC-A (0.0005N potassium hydrogen phthalate)

Dilute 100.0 ml of stock to 1 liter with distilled, deionized water. (Theoretical acidity = $500\text{ }\mu\text{eq/l}$ or 25 mg/l acidity as CaCO_3).

4.12. Quality Control Solution B - Stock (0.02N H_2SO_4)

Dilute 1 ampoule of N/50 concentrate to 1 liter according to package directions.

4.13. Quality Control Solution B - Working QC-B (0.0002N H_2SO_4)

Dilute 10.0 ml of stock to 1 liter with distilled, deionized water. (Theoretical acidity = $200\text{ }\mu\text{eq/l}$ or 10 mg/l acidity as CaCO_3).

5. Procedure

REFER TO THE MANUFACTURER'S MANUAL FOR GENERAL OPERATING PROCEDURES OF THE TRS-80 OR PET 2001 MICROCOMPUTER, ABU 80 AUTOBURETTE AND pHM 84 pH METER.

5.1. Preparation of the ABU 80 Autoburette

Fill the reservoir bottle of the ABU 80 autoburette with the 0.01N sodium hydroxide working titrant.

Protect the titrant from carbon dioxide absorption by using a carbon dioxide absorbent (e.g. Ascarite). Ensure that the Ascarite column is not blocked and that air may pass freely. If the reservoir bottle has been refilled or the titrant has been changed ensure that the burette delivery system is flushed with fresh titrant by pressing the FLUSH button on the autoburette. The normal operating conditions for the autoburette are:

- Power ON.
- Speed control set at 160.
- Ready light ON.
- MAN/AUTO control in the MAN position.
- Volume control in the 1/1 position.

5.2. Standardization of the pHM 84 pH Meter

REFER TO THE METHOD OF pH MEASUREMENT FOR A MORE DETAILED DISCUSSION.

- 5.2.1. Ensure that the electrodes are in good condition; that the reference filling solution is at the required level (replenish if necessary); and that the electrode filling hole is open to the atmosphere.
- 5.2.2. Pour an appropriate volume of pH 4.00 and 7.00 buffer into beakers, each containing a magnetic stirring bar.
- 5.2.3. Set the pH meter temperature dial to the appropriate temperature.
- 5.2.4. Set the ISO pH Control of the meter to 7.
- 5.2.5. Place the electrode in buffer 7.00 solution and while stirring, use the BUFFER CONTROL dial to adjust the pH meter reading to 7.000 ± 0.005 .
- 5.2.6. Remove the electrode from the buffer 7.00 solution and rinse with distilled water.
- 5.2.7. Place the electrode in buffer 4.00 solution and while stirring use the SLOPE CONTROL dial to adjust the pH meter reading to 4.000 ± 0.005 .
- 5.2.8. Check the adjustment several times by repeating steps 5.2.5 to 5.2.7 until no further adjustment is necessary and record the final SLOPE reading.
- 5.2.9. Read pH 6.86 and 4.01 buffers as Quality Control solutions and record the pH readings for each.

5.3. Preparation of the TRS-80 Microcomputer system

- 5.3.1. Turn on the microcomputer system in the following order:
- Interface/Power supply box (in-house design).
 - TRS-80 Keyboard.
 - TRS-80 Video Display.
 - TRS-80 Line Printer.
 - TRS-80 Expansion Interface.
- 5.3.2. When MEMORY? shows on the screen, press ENTER on the keyboard and READY> will appear. If the MEMORY? or READY> fails to appear hold the BREAK key and press the RESET button on the back of the keyboard.
- 5.3.3. Load the appropriate program using the CTR 80 recorder, or if available, using the TRS-80 disc drive. Refer to the User's Manual for details regarding loading procedure.
- 5.3.4. Enter RUN and the appropriate data via the TRS-80 Keyboard as requested. When START? appears, press ENTER to start the titration.

Preparation of the PET 2001 Microcomputer System

- 5.3.5. Turn on the microcomputer system in the following order:
- Interface/power supply box (in-house design)
 - PET Keyboard/Video
 - PET Line Printer
 - PET Disc Drive
- 5.3.6. Load the Appropriate program via the disc drive. Refer to the User's Manual for details regarding loading procedure.
- 5.3.7. Once the program is loaded type in RUN and press RETURN. Enter the appropriate data as requested on the screen, pressing RETURN after each entry.
- 5.3.8. **Comments on use of both microprocessors**

To interrupt execution at any time, use the BREAK key on TRS-80 or SHIFT and RUN/STOP keys on PET. To resume operation enter RUN then enter requested paramters.

If, on the PET, RETURN is hit without an input character, program execution is halted. To continue enter CONT.

There is no need to re-load the program on either system unless the power has been turned off.

A maximum of 150 data points are permitted for each titration.

The programs calculate inflection points during the titration. These appear on the display designated by an asterisk (*). These points are interpolated on the titration curve and, therefore, pH and volume appear "out of order" with respect to the remainder of the points. The inflection points are not printed in the titration table.

5.4. Standardization of Working Titrant ($\approx 0.01N$ sodium hydroxide).

- 5.4.1. Pipette 10.0 ml of standard potassium hydrogen phthalate solution ($5.00 \times 10^{-3}N$) into a 200 ml beaker. Adjust the volume to 100 ml with distilled, deionized water. Titrate with working sodium hydroxide titrant using the microcomputer controlled system. Repeat the analysis for a duplicate sample of standard potassium hydrogen phthalate. Calculate the normality of the working sodium hydroxide titrant according to the procedure outlined in Section 6.
- 5.4.2. Interrupt the execution of the program after the standardization procedure and re-enter the appropriate data as requested on the Video Screen. The normality of base required in the program is that calculated for the working sodium hydroxide titrant from standardization against potassium hydrogen phthalate.

5.5. Titration of Samples

- 5.5.1. Pipette an appropriate volume of sample or Quality Control Standard (usually 100 ml) into a clean beaker containing a stirring bar and place on a stirrer. Set stirrer at a rate which does not create a vortex. An aliquot of each working QC-A and QC-B solution should be titrated daily and the data recorded.
- 5.5.2. Lower the electrode assembly into the sample ensuring that the electrode tip is clear of the stirring bar. In order to achieve pH stability it is necessary to adjust the relative heights of the delivery tip and pH electrode. Place the delivery tip as close as possible to the stirring bar and keep the electrode near the surface. Ensure that the porous plug of the electrode is below the surface of the sample.
- 5.5.3. After each titration is complete, press N for new sample or P for page feed and reply Y(yes) or N(no) for the same or new conditions when requested. Press BREAK on the TRS-80 or SHIFT and RUN/STOP on the PET when all samples are complete.
- 5.5.4. Power-off the equipment in the reverse order to the power-on sequence described in section 5.3.1. or 5.3.5.
- 5.5.5. Replace the rubber band over the filling hole of the electrode and store the electrode in the solution recommended by the manufacturer (distilled water for Ingold electrodes or pH 7 buffer for Radiometer electrodes).

6. Calculation and Reporting

The normality of the working sodium hydroxide titrant is determined by duplicate (minimum) standardization against 10 ml of $5.00 \times 10^{-3}N$ potassium hydrogen phthalate solution as follows:

$$N_{\text{NaOH}} = \frac{10.00 \times 0.005}{v}$$

Where:

v = Gran endpoint volume (in ml) given in the titration table printout (i.e. volume of sodium hydroxide titrant needed to raise the pH of the standard potassium hydrogen phthalate solution to its inflection point.

$$\text{mg/l CO}_2 = \frac{V \times N_{\text{NaOH}}}{V_s} \times 44,000$$

Results are reported as mg/l CO₂ to three significant figures.

Where:

V = titrant volume (ml) (i.e. volume of titrant required to reach the specified endpoint)

N_{NaOH} = titrant normality

V_s = sample volume (ml)

7. Precision and Accuracy

The automated titration method exhibits a standard deviation of 0.06 mg/l carbon dioxide in the 0 - 10 mg/l range. Some sample duplicates may deviate from this criteria due to the presence of particulate matter.

8. Bibliography

- 8.1. Ministry of the Environment (1975). Outline of Analytical Methods. Laboratory Services Branch, Rexdale, Ontario.
- 8.2. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater. 13th ed. APHA, Washington, D.C. 50-52.

THE DETERMINATION OF CHLORIDE

Chloride is widely distributed in the environment and is contributed through the weathering of primary minerals. It is usually found in combination with sodium, but is occasionally associated with calcium or magnesium. Chloride is present in all natural waters, varying in concentration from less than a few mg/l to several thousand mg/l in some ground waters. Oceanogenic salinity is also a major source of chloride, and rainwater exhibits higher chloride concentrations in areas close to the ocean than in areas further inland. In Canada, the average chloride concentration in precipitation is a few tenths of a mg/l.

Chloride is generally recognized as an essential element for plant growth. Levels in dried vegetation, may vary widely with species, although concentrations of between 0.3 and 1.0% are commonly found. Consequently, a detailed knowledge of specific plant requirements is necessary for the correct interpretation of analytical data. Chloride tends to accumulate in the older tissues of plants and if present in toxic amounts may result in leaf tip burn.

Winter road salting is a primary source of chloride damage to vegetation, either through vehicular spray or through the contamination of surface or ground waters. Sewage and industrial wastes containing chloride in concentrations exceeding that of the receiving stream, and the leaching of salt stockpiles may also contribute to chloride contamination of the environment. Chloride salts are poorly retained in soils and therefore when introduced to the soil move readily into the water system. Ministry guidelines for Ontario suggest that chloride levels in drinking water should not exceed 250 mg/l.

Sample Handling and Preservation

Water, Sewage, Industrial Wastes

Glass or polyethylene bottles are suitable for sample collection. Glass containers are not recommended for precipitation samples due to the acidic nature of many of the samples and the low levels of chloride being determined.

Since the chloride ion does not readily enter into chemical reactions or form many insoluble salts, chloride solutions are stable indefinitely and require no preservatives.

Vegetation

Samples are collected in perforated polyethylene bags and are refrigerated as soon as possible after collection. When dried and ground, samples may be stored in glass jars under dry conditions for several years without significant deterioration.

Soil and Sediment

Soil and sediments may be collected in pomade jars. Samples are air dried and ground to pass through a 2 mm sieve.

Selection of Method

A variety of methods are employed for the determination of chloride depending upon the sample matrix and the levels of chloride present. Method A, a potentiometric titration, is selected for the analysis of drinking water, sewage and industrial wastes, when precise determinations are required for samples containing moderate amounts of chloride. Method B, an automated colorimetric method is sensitive to low concentrations of chloride and is applied to river and lake (surface water) samples. Method C, an automated ion chromatographic technique, was designed specifically for the determination of the major anionic constituents of precipitation samples. The precision and accuracy of this technique is very satisfactory and the system is considerably more sensitive to sulphate, chloride and fluoride than colorimetric procedures. A summary of the ion chromatographic method is provided herein, with a complete description appearing in The Determination of Sulphate, Method B. Method D is an alkali-fusion colorimetric method applied to vegetation, soil and sediment samples. Vegetation samples may also be analysed by X-ray fluorescence, a complete description of which is given in The Determination of Trace Metals by Atomic Spectroscopy.

CHLORIDE

Automated Potentiometric Titration Method A

SUMMARY

Matrix.	This method is used routinely for Cl^- determinations on domestic waters, sewages and industrial wastes.
Substance determined.	Chloride ion, Cl^- .
Interpretation of results.	The results are reported as mg/l Cl^- although bromide and iodide are also included when present.
Principle of method.	An aliquot of sample is titrated with silver nitrate to a predetermined endpoint. An automatic titrator with a silver-silver chloride, non-calomel reference, electrode system is used.
Time required for analysis.	10 minutes are required for a single analysis. Approximately 150 tests can be performed in a day.
Range of application.	Minimum 0.5 mg/l, maximum 400 mg/l. Higher levels are determined by dilution.
Standard deviation.	Based on within run duplicates for the Radiometer system standard deviations are 0.31 in the 0 - 80 mg/l Cl^- range; 0.25 in the 80 - 200 mg/l Cl^- range; 0.52 in the 200 - 400 mg/l Cl^- range.
Accuracy.	Recoveries of two Quality Control solutions were 100% and 103% respectively.
Detection criteria.	0.5 mg/l.
Interferences and shortcomings.	<p>Bromide, cyanide and iodide register as equivalent chloride concentrations. Chromate and dichromate and ferric ion interfere if present in amounts greater than the amount of chloride. Phosphate, thiocyanate, sulphide, sulphite, hydroxide and thiosulphate are potential interferences; however, their concentrations are usually insignificantly low.</p> <p>Sulphide, sulphite, and hydroxide interference can be eliminated by acidification to pH 2 or less with concentrated nitric acid followed by vigorous aeration for 5 minutes. Thiosulphate interference is eliminated by treatment with alkaline hydrogen peroxide.</p>

**Minimum volume
of sample.**

75 ml for chloride levels up to 400 mg/l. Less volume is required for higher concentrations.

**Preservation and
sample container.**

Glass or polyethylene containers are suitable for sample collection. No preservative is necessary.

**Safety
considerations.**

Silver nitrate is poisonous and inhalation of the powder or contact with the solution must be avoided.

CHLORIDE

Automated Potentiometric Titration Method A

1 Introduction

An aliquot of sample is automatically pipetted and titrated with silver nitrate to a pre-determined endpoint using an automatic titrator with a silver-silver chloride and non-calomel reference electrode system. The addition of silver nitrate to a sample containing chloride ion results in the precipitation of silver chloride with a corresponding change in the emf between the electrodes. A Radiometer ATS-1 system is currently used in the main laboratory while the regional laboratories use a Fisher Titralyzer system. Quality control data is given only for the Radiometer ATS-1 system.

2. Interferences and Shortcomings

Bromide, iodide and cyanide register as equivalent chloride concentrations. Phosphate and thiocyanate may also interfere. These interfering substances are present in extremely small quantities in natural waters; however, somewhat higher levels may occur in industrial waste waters. Cyanide can be removed by acidification of the sample.

NOTE: Samples containing cyanide release hydrogen cyanide (HCN) when acidified. This is an extremely toxic gas and the procedure must be performed in a fumehood.

Chromate, dichromate, and the ferric ion interfere if present in amounts greater than the amount of chloride. Reduction to chromic and ferrous ions respectively, can eliminate these interferences.

Sulphides, sulphite, and hydroxide interferences are removed by sample acidification with concentrated nitric acid to a pH of 2 or less followed by sample aeration for 5 minutes.

Thiosulphate interference can be eliminated by oxidation to sulphate with alkaline hydrogen peroxide.

3. Apparatus

3.1. Fisher Titralyzer TM Automatic Titrator with:

- 3.1.1. Indicator electrode, silver-silver chloride electrode responding to changes in the chloride ion concentration.
- 3.1.2. Reference electrode, silver-silver chloride, internal with silver sulphate electrolyte.
- 3.1.3. Berzelius Beakers, 250 ml capacity, 50 mm diameter with 1/8" flattened rims.

NOTE: Beakers must be scrubbed with a mop or sponge to prevent the build-up of a film of AgCl on the walls.

OR

3.2. Radiometer ATS-1 Autopipetting Titration System.

3.2.1. Indicator electrode, silver-silver chloride electrode responding to changes in chloride ion concentration.

3.2.2. Reference electrode, mercury-mercurous sulphate type, potassium sulphate electrolyte.

4. Reagents

4.1. Sodium chloride (NaCl) reagent grade crystals.

4.2. Silver nitrate (AgNO_3) reagent grade crystals.

4.3. Potassium chromate (K_2CrO_4) reagent grade.

4.4. Acetone ($(\text{CH}_3)_2\text{CO}$) reagent grade.

4.5. Nitric Acid (HNO_3), reagent grade.

4.6. Calcium Carbonate (CaCO_3), reagent grade.

4.7. Potassium Chromate Indicator

Dissolve 5 g potassium chromate in 25 ml distilled water. Add silver nitrate until a definite red precipitate is formed. Let stand for 12 hours; filter and dilute with distilled water to a final volume of 100 ml.

4.8. Standard Chloride Solution (500 mg/l)

In a volumetric flask, dissolve 0.8241 g oven dried (140°C) sodium chloride in distilled water and dilute to 1 liter in a volumetric flask.

Fisher Titralyzer System

4.9. Standard Silver Nitrate Reagent (0.0141N)

Dissolve 19.5516 g silver nitrate in distilled water and dilute to 8 liters. Store away from direct sunlight in a brown glass bottle. Standardize according to 5.1.

5.1. After standardization 1 ml of this solution = 0.500 mg Cl.

Radiometer ATS-1 System

4.10. Standard Silver Nitrate Reagent (0.05641N)

Dissolve 19.16566 ± 0.00005 g silver nitrate in distilled water and dilute to 2 liters. Standardize as in 5.1.

NOTE: Silver nitrate is poisonous and inhalation of powder or contact with the solution must be avoided.

4.11. Acetone-Water-Nitric Acid Reagent

Dilute 15 ml concentrated nitric acid to 1 liter with distilled water. Dilute the resulting solution to 2 liters with reagent grade acetone.

4.12. Nitric Acid Wash Solution

Dilute 40 ml concentrated nitric acid and 10 ml of sodium chloride stock solution (or the equivalent) to 2 liters with distilled water.

4.13. Sodium Chloride Stock Solution (1000 mg/l Cl)

Dissolve 1.64846 ± 0.00005 g sodium chloride in distilled water and dilute to 1 liter.

4.14. Sodium Chloride Working Standard (10 mg/l Cl)

Dilute 10 ml sodium chloride stock solution to 1 liter with distilled water.

4.15. Calibration Standards:

Dissolve 0.65938 ± 0.00005 g sodium chloride (dried at 140°C for 2 hours and cooled in a desiccator) in distilled water and dilute to 1 liter with distilled water. This gives a 400 mg/l Cl solution.

Dilute 25 ml of the 400 mg/l solution to 1 liter with distilled water to give a 10 mg/l Cl solution.

4.16. Quality Control Stock Solution

Dissolve 2.8024 ± 0.0001 g sodium chloride (dried as in 4.15) in distilled water and dilute to 1 liter. Use a different batch of sodium chloride from the one used for preparation of reagents 4.13 and 4.15.

4.17. Quality Control Working Solutions

QC-A: Dilute 100 ml quality control stock solution to 2 liters with distilled water to give a chloride concentration of 170 mg/l.

QC-B: Dilute 10 ml quality control stock solution to 2 liters with distilled water to give a chloride concentration of 10 mg/l.

NOTE: Quality control solutions used for the Fisher Titralyzer system are chosen by the regional laboratory so that the chloride concentrations are at about 20% and 80% of scale. The sodium chloride used is from a different batch than the one used for preparation of the working standards.

4.18. Reference Electrode Filling Solution

Dissolve 20 g potassium sulphate in distilled water and dilute to 100 ml.

5. Procedure

5.1. Standardization of the Standard Silver Nitrate Reagent

Pipette 20 ml standard chloride solution into 3 white casseroles and dilute to about 50 ml with distilled water. In a fourth casserole add 50 ml distilled water and sufficient calcium carbonate powder to approximate the white background color at the endpoint in a non-blank titration. Add 5 drops of potassium chromate indicator to each sample and titrate with the silver nitrate until the first sign of the brick red precipitate persists. The normality of the silver nitrate solution is given by:

$$\text{Normality, AgNO}_3 = \frac{500 \times 20.0}{35450 \times (V_S - V_B)}$$

Where:

V_S = Volume of silver nitrate required to reach the endpoint with 20.0 ml of standard chloride solution.

V_B = Volume of silver nitrate required to titrate the blank to the equivalent endpoint.

The normality of the AgNO_3 solution must be adjusted to 0.0141N before use in the Fisher Titralyzer, or 0.05641N before use in the Radiometer ATS-1.

5.2. Electrode Care – All Systems

- 5.2.1. Clean the silver billet electrode with an abrasive cleanser such as Ajax. Clear the porous tip of the reference electrode by polishing on crocus cloth.
- 5.2.2. Ensure that potassium sulphate crystals are present in the reference electrode and that the crystals do not form a solid mass blocking the porous plug. Such a block may be broken up by warming the electrode in hot water and tapping gently.
- 5.2.3. Condition the silver billet electrode by titrating two samples containing 25 mg/l of Cl^- or more (e.g. Lake Ontario water).
- 5.2.4. Drifting response to the in-run control solutions may be the result of dirty electrodes. The tip of the reference electrode may be cleared by polishing on crocus cloth at any time. The silver billet electrode may be wiped with a Kimwipe to remove gross deposits without restandardizing. If the silver billet is cleaned as in 5.2.1 above, recondition and restandardize before proceeding with samples.

5.3. Pretreatment for Landfill Leachates, Sludges, Sewages and Sulphide-Bearing Waters.

- 5.3.1. Centrifuge or filter a portion of sample to provide 12 ml clarified supernatant.
- 5.3.2. Rinse sample cup with sample and fill to 12 ml mark.
- 5.3.3. Add 2 drops concentrated nitric acid and aerate for a few seconds.
- 5.3.4. Using pH paper check that sample is strongly acidic. If not, repeat 5.3.3 and re-test.
- 5.3.5. Aerate for 5 minutes. Analyze as in 5.4 or 5.5.

5.4. Analysis by the Fisher Titralyzer System

REFER TO MANUFACTURER'S MANUAL FOR OPERATING INSTRUCTIONS.

NOTE: Refer to appropriate laboratory quality control record for control limits and solution preparation.

- 5.4.1. With the MV-pH selector switch in the MV position and the instrument operation selector in the zero position, use the zero adjust

control to null the current meter. Set the endpoint selector to MV and adjust to a predetermined endpoint of 420 mv. Rotate the instrument operation selector to USE prior to starting the run.

- 5.4.2. List samples to be analyzed. Pipette 50 ml aliquots of each sample or specified aliquots of control solutions into separate beakers.
- 5.4.3. Add 2 ml acetone to each beaker. The acetone serves to disperse the precipitate in the solution and prevents it from collecting on the electrodes.
- 5.4.4. Place the sample beakers in the turntable. Start the automatic operation by lowering the electrodes into the first beaker.
- 5.4.5. Replace titrated samples with a new series after each cycle is completed. Advance the printout tape a few spaces after each cycle. Record results.

5.5. Analysis by the Radiometer ATS-1 System

REFER TO MANUFACTURER'S MANUAL FOR GENERAL OPERATING INSTRUCTIONS.

5.5.1. Operating Conditions

ATS-1

Pressure: 70 - 80 psig
Stirring: 800
Sample: 10
Reagent 1: 2
Reagent 2: 0

Titration TTT-2:

Temperature °C: inactive
Buffer: inactive
Range: x1 mv
Delay Shut-off: 3
Titration: upscale
Proportional band: 1.0

Autoburette:

Speed: 20
Increments: off
Auto Refill: in
All others: out

- 5.5.2. Ensure that there is sufficient printer paper, silver nitrate titrant, acetone-water-nitric acid reagent, that the waste container is empty, the dehumidifier is dry and that the sample pipette reaction cup, stirrer and titrant delivery tip are clean.

5.5.3. Calibration

5.5.3.1. Titrate two 10 mg/l Cl calibration standards.

5.5.3.2. Continue to titrate the 10.0 mg/l standards adjusting the mv endpoint to yield a titration of 0.050 ± 0.002 ml.

- 5.5.3.3. Record mv setting (this should be 115 mv \pm 30 mv). Record titration volume.
- 5.5.3.4. Titrate 400 mg/l chloride standard. Record result. This must be \pm 1.5% of the true value.
- 5.5.3.5. Titrate the nitric acid wash.
- 5.5.3.6. Titrate QC-A and QC-B and ensure that these fall within the quality control limits.
- 5.5.4. Rinse sample cups twice with a small portion of the solution to be analyzed and fill to the 12 ml mark with sample. Samples which underwent the pretreatment specified in 5.3 will already be at this stage.
- 5.5.5. After calibration is complete run groups of: (nitric acid wash; blank; working standard; 15 samples)
 After every 4 groups of the above, run: (nitric acid wash; 400 mg/l Cl calibration standard, blank).
 At the end of the run, titrate: (nitric acid wash; 400 mg/l Cl calibration standard; blank).
 Record titrations of the blank, working standard and 400 mg/l calibration standard on the control sheets.
- 5.5.6. Stop run and clean electrode if: the blank titration exceeds 0.010 ml, the working standard has an error of greater than \pm 0.010; or the 400 mg/l calibration standard has an error of greater than \pm 1.5%. After cleaning re-run: nitric acid wash; blank; 10 mg/l working standard; 400 mg/l calibration standard.
- 5.5.7. After the run, store electrodes in a beaker of saturated potassium sulphate solution.

6. Calculation and Reporting

Fisher Titralyzer: Results are reported as mg/l chloride:

$$\text{mg/l Cl} = \frac{a \times N \times 354.50}{\text{ml sample}}$$

Where:

a = ml of silver nitrate required to titrate the sample.

N = normality of the silver nitrate.

Radiometer ATS-1:

The silver nitrate volume used for the titration is printed out to the nearest 0.001 ml. Chloride concentration in the sample is calculated as follows:

$$\text{mg/l Cl} = 200 \times \text{volume of silver nitrate.}$$

7. Precision and Accuracy

Based on within-run duplicate samples in the 0 - 400 mg/l Cl range standard deviations are: 0.31 in 0 - 20% of the range; 0.25 in 20 - 50% of the range and 0.52 in 50 - 100% of the range.

Recoveries of two Quality Control solutions were 100% and 103% respectively.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th ed., APHA, Washington, D.C. 306-309.
- 8.2. Fisher Titralyzer, Instruction Manual.
- 8.3. U.S. Geological Survey (1970). Study and interpretation of the chemical characteristics of natural water. Water Supply Paper No. 1473. U.S. Department of Interior. 363P.

CHLORIDE

Automated Colorimetry Method B

SUMMARY

Matrix.	This method is used routinely for Cl^- determinations on surface water samples.
Substance determined.	Chloride ion, Cl^- .
Interpretation of results.	The results are reported as mg/l Cl^- although bromide and iodide are also included when present.
Principle of method.	Chloride ions combine with mercuric thiocyanate to form an undissociated salt, mercuric chloride, and release thiocyanate ions which then complex with ferric ion to produce a colored solution. The absorbance of the colored solution is proportional to the original concentration of chloride ion in the sample. Two analytical ranges are obtained from the output signal of the colorimeter.
Time required for analysis.	Each analysis requires 3 or 4 minutes. Several hundred tests can be completed in a day.
Range of application.	Low range 0.10 - 10.0 mg/l High range up to 50 mg/l.
Standard deviation.	Based on within-run duplicate samples standard deviations are: 0.051 in the 0.1 - 2 mg/l Cl^- range; 0.056 in the 2 - 5 mg/l Cl^- range; 0.065 in the 5 - 10 mg/l Cl^- range; 0.175 in the 10 - 30 mg/l Cl^- range and 0.293 in the 30 - 50 mg/l Cl^- range. Recoveries of Quality Control solutions were 101% for the low range and 103% and 107% for the high range.
Detection criteria.	0.084 mg/l Cl^- .
Interferences and shortcomings.	Bromide and iodide register as equivalent chloride concentrations. Thiocyanate and thiosulphate also increase the measured chloride concentration.

**Minimum volume
of sample.**

50 ml required for both ranges.

**Preservation and
sample container.**

Glass or polyethylene containers are suitable for sample collection and storage. No preservative is necessary.

**Safety
considerations.**

Mercury thiocyanate is poisonous. Waste solutions containing soluble mercury salts should be retained for mercury removal prior to disposal.

CHLORIDE

Automated Colorimetry Method B

1 Introduction

Chloride combines with mercuric thiocyanate to produce an undissociated salt, mercuric chloride. This reaction liberates thiocyanate which then complexes with the ferric ion under acidic conditions to produce a reddish colored solution. The absorbance of the solution is proportional to the original concentration of chloride ion in the sample. A reference stream is operated parallel to the color producing stream to minimize the effects of sample color. Except for the replacement of mercuric thiocyanate reagent with distilled, deionized water, the reference stream is identical to the color producing stream. Two analytical ranges are obtained from the output of the AAll colorimeter.

2 Interferences and Shortcomings

Bromide and iodide register as equivalent chloride concentrations. Thiosulphate interference can be eliminated by oxidation to sulphate with alkaline hydrogen peroxide. Thiocyanate interference can be confirmed by the addition of ferric ion. Analysis can be completed by differential colorimetric methods.

3 Apparatus

- 3.1. AutoAnalyzer System (Technicon or equivalent), using 460 or 480 nm interference filters and 50 mm flow cells. See Figure 1.

4 Reagents

- 4.1. Sodium chloride (NaCl), reagent grade crystals.
- 4.2. Mercuric thiocyanate ($\text{Hg}(\text{SCN})_2$) reagent grade crystals.
- 4.3. Ferric ammonium sulphate ($\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$), reagent grade crystals.
- 4.4. Nitric Acid (HNO_3) concentrated, reagent grade.

4.5 Ferric Ammonium Sulphate Solution

Dissolve 180 g ferric ammonium sulphate in 1400 ml distilled, deionized water. Carefully add 400 ml concentrated nitric acid. Dilute to 2 liters with distilled, deionized water and filter before use.

4.6 Mercuric Thiocyanate Solution

Prepare a saturated solution by adding 3.5 g mercuric thiocyanate to 4 liters distilled, deionized water. Stir overnight and filter before use.

NOTE: Mercuric thiocyanate is poisonous. Handle with care.

4.7. Chloride Stock Solution (1000 mg/l Cl)

Dissolve 1.6485 g sodium chloride (oven dried and cooled in a desiccator) in distilled, deionized water and dilute to 1 liter.

4.8. Quality Control Stock Solution (2000 mg/l Cl)

Dissolve 3.2970 g sodium chloride (dried as above) in distilled, deionized water and dilute to 1 liter. (Use a different batch of sodium chloride than that used for the preparation of 4.4.)

4.9. Chloride Working Standards (0 – 50 mg/l range)

Using volumetric flasks, dilute 40 ml and 8 ml chloride stock solution to 1 liter with distilled, deionized water. This gives standards with chloride concentrations of 40 mg/l and 8 mg/l respectively.

4.10. Chloride Working Standards (0 – 10 mg/l range)

Dilute 8 ml and 2 ml chloride stock solution to 1 liter with distilled, deionized water. This gives standards with chloride concentrations of 8 mg/l and 2 mg/l respectively.

4.11. Quality Control Standards (0 – 50 mg/l range)

QC-A: Dilute 50 ml quality control stock solution to 4 liters with distilled, deionized water to give a chloride concentration of 25 mg/l.

QC-B: Dilute 10 ml quality control stock solution to 4 liters with distilled, deionized water to give a chloride concentration of 5 mg/l.

4.12. Quality Control Standards (0 – 10 mg/l range)

QC-C: Prepare as in QC-B solution 4.11.

QC-D: Dilute 8 ml quality control stock solution to 4 liters with distilled, deionized water to give a chloride concentration of 4 mg/l.

4.13. Daily Sensitivity Checks (0 – 50 mg/l range)

Dilute 40 ml and 8 ml chloride stock solution to 1 liter with distilled, deionized water to give chloride concentrations at 80% and 16% of range.

4.14. Daily Sensitivity Checks (0 – 10 mg/l range)

Dilute 8 ml and 2 ml chloride stock solution to 1 liter with distilled, deionized water to give chloride concentrations at 80% and 16% of range.

5. Procedure

REFER TO MANUFACTURER'S MANUAL FOR GENERAL OPERATING INSTRUCTIONS FOR THE AUTOANALYZER SYSTEM.

- 5.1 Set the AutoAnalyzer into operation using cleaning, set-up and checking procedures appropriate to the manifold is illustrated in Figure 1.
- 5.2 Collect the samples and group them according to the bench sheet.
- 5.3 Load the sample module such that each run includes the following units:
 - Set of calibration standards: 40, 8, 2 mg/l
 - Distilled water blank (day's supply): Bl
 - Quality control samples: QC-A, QC-B, QC-C, QC-D
 - Long term blank: LTBl
 - Sensitivity checks: L, M, H

The basic loading sequence is 10 samples, Bl, 10 samples, L, M, H, Bl.
- 5.4 Calibrate the AutoAnalyzer system using calibration standards. Record the standard calibration setting and check to ensure that it has not changed unduly.
- 5.5 Check the calibration by analysing the quality control solutions. Record these values as well as the long term blank. For each range calculate the sums and differences, eg., QC-A plus QC-B and QC-A minus QC-B, etc. Check to ensure that the calculated values are within the specified control limits before proceeding with the run.
- 5.6 Monitor the sensitivity throughout the run to determine if within-run sensitivity corrections are required.
- 5.7 Run at least 4 pairs of duplicates randomly distributed throughout the run.
- 5.8 Read sample peak heights and convert to concentration values.

6. Calculation and Reporting

Results are reported as mg/l Cl^- .

Report results to 2 significant figures for both ranges.

7. Precision and Accuracy

Standard deviations for within-run duplicate samples are given as follows:

Concentration Range	S_{ld}	S_{md}	S_{hd}
0 - 50 mg/l	-	0.175	0.293
0 - 10 mg/l	0.051	0.056	0.065

Where:

S_{ld} = standard deviations for 0 - 20% of range.

S_{md} = standard deviations for 20 - 50% of range.

S_{hd} = standard deviations for 50 - 100% of range.

Recoveries for Quality Control Solutions were 101% for the low range and 103% and 107% for the high range.

8. Bibliography

- 8.1. Florence, T.M. and Farrar, Y.J. (1971). Spectrophotometric determination of chloride at the parts per billion level by mercury (II) thiocyanate method. *Analytica Chimica Acta*, **54**: 373-377.
- 8.2. Zall, D.M., Fisher, D.M. and Garner, M.Q. (1956). Photometric determination of chlorides in water. *Analytical Chemistry*, **28**: 1665-1668.

CHLORIDE

Automated Ion Chromatography Method C

SUMMARY

Matrix.	This method is used routinely for chloride determinations on precipitation samples.
Substance determined.	Chloride ion, Cl^- .
Interpretation of results.	Results are reported as mg/l Cl.
Principle of method.	Chloride is separated from other anions in the sample by using a column packed with ion exchange resin and an eluent composed of a mixture of sodium bicarbonate and sodium carbonate. Chloride is converted to an acid form by ion exchange and its concentration is determined from the conductivity of the hydrochloric acid produced. Manually drawn calibration curves are used.
Time required for analysis.	The time required for analysis depends upon eluent concentration and flow, the size of the separator column and the number of ions being determined. Assuming chloride, nitrate, fluoride and sulphate are determined about 6 minutes per sample are required.
Range of application.	a) 0.03 - 1.20 mg/l Cl. b) 1.20 - 1.50 mg/l Cl.
Standard deviation.	0.017 mg/l.
Accuracy.	Recoveries of Quality Control solutions were 100% and 99%.
Detection criteria.	0.028 mg/l.
Interferences and shortcomings.	Interferences occur when two ions have a similar retention time and when one ion is present in much higher concentrations than the other. The higher and broader peak will mask the lower concentration peak. This, however, is not usually a problem with chloride.

Minimum volume of sample.	15 ml if sampling procedure is automated.
Preservation and sample container.	Polyethylene bottles are recommended for sample storage. No preservative is necessary.
Safety considerations.	Regular precautions should be taken when handling sodium hydroxide and concentrated acids. The pressure should not be allowed to exceed 500 or 600 psi. The machine should be operated with the column doors closed in case pressure increases and explosion occurs.

THE AUTOMATED ION CHROMATOGRAPHY PROCEDURE IS DESCRIBED IN "THE DETERMINATION OF SULPHATE" METHOD B.

CHLORIDE

Alkali - Fusion - Semi-Automated Colorimetry Method D

SUMMARY

Matrix.	This method is used for the analysis of vegetation, soil and sediment samples.
Substance determined.	Chloride ion, Cl.
Interpretation of results.	Chloride concentrations are reported as % of dried sample.
Principle of method.	Oven dried vegetation samples and air dried soil samples are treated with an excess of calcium hydroxide solution, to bind volatile halides while ashing. Treated samples are oven dried and ashed in a muffle furnace to convert all halide present into organic form. Ashed samples are fused with excess sodium hydroxide, to convert all chloride into a soluble form. Fused samples are dissolved in aqueous solution, acidified with nitric acid and reacted with mercuric thiocyanate. The chloride forms unionized and insoluble mercuric chloride and liberates an equivalent quantity of thiocyanate ion. This is reacted with ferric ion, to produce an intense red color which is measured colorimetrically at 460 nm. The absorption of the unknown sample is compared to that of known standards and the concentration calculated.
Time required for analysis.	For a single operation including all sample weighings and subsequent steps, up to 250 samples per week can be processed.
Range of application.	Based on 1 g of dried vegetation and .02 g soil or sediment, the routine working range in terms of dried sample is from 0.02% to 0.50% chloride. The upper range may be extended by further dilution or by a reduction in sample size.
Standard deviation.	$\pm 7.9\%$ based on 303 pairs of duplicates.
Accuracy.	Calibration is maintained by 2 long-term standards QC-A and QC-B such that the measured value of these standards is within 2 standard deviations for the long-term mean value of these standards.
Detection limit.	The detection limit is 0.2% chloride.

Minimum volume of sample.

1 g dried vegetation; 0.2 g dried soil or sediment. However, for the sample to be representative, a homogenized dried vegetation sample of 20 g (about 200 g of fresh material) is required. A soil sample of about 10 g is recommended.

Interferences and shortcomings.

Interferences with the colorimetric procedure are similar to those described in Method B, however, no significant interferences have been observed in the analysis of vegetation and soils.

Preservation and sample container.

Collect vegetation samples in perforated polyethylene bags and refrigerate. Oven dry, grind to less than 80 mesh and store in glass jars. Air dry and grind to less than 2 mm.

Safety considerations.

Apart from the actual weighing of samples, almost all other operations involve the manipulation of strong acids and bases. Splash-protective eye shields should be worn routinely. It is stressed that normal glasses or safety glasses designed for explosive situations are not adequate protection against accidental spillages.

Fusion operations with caustic alkalis must not be undertaken without the protection of a full face shield.

CHLORIDE

Alkali-Fusion-Semi-Automated Colorimetry Method D

1. Introduction

A sample is treated with calcium hydroxide, ashed, fused with sodium hydroxide, and dissolved in an aqueous solution. An aliquot of the solution is automatically sampled, diluted with distilled water and acidified with nitric acid. Excess mercuric thiocyanate and ferric ammonium sulphate are added and the resulting ferric thiocyanate red color is measured at 460 nm. Absorption of the unknown sample is then compared to that of known standards and the chloride concentration is calculated.

2. Interferences and Shortcomings

Bromide and iodide are potential interferences, as mentioned in Method B. However, these interferences have not been encountered in the analysis of soil and vegetation samples.

3. Apparatus

3.1. AutoAnalyzer AAI system comprised of the following modules:

- 3.1.1. sampler
- 3.1.2. proportioning pump
- 3.1.3. heating bath, heating to 170°C equipped with thermostatic control and microdistillation head
- 3.1.4. colorimeter fitted with 460 nm filters and a 1.5 cm flow cell
- 3.1.5. voltage regulator
- 3.1.6. chart recorder.

3.2. Pump tubing and assorted manifold glassware as in Figure 2.

3.3. Muffle furnace and controller with temperature range to 1000°C.

3.4. Drying oven heating to 105°C.

3.5. Balance, top loading, accurate to ± 1 mg with tare.

3.6. Nickel crucibles, 50 ml, low form.

3.7. Flasks, volumetric, plastic, assorted sizes.

3.8. Pipettes, volumetric, assorted sizes.

3.9. Test tubes, 85 x 14 mm.

3.10. Test tube rack, 40 tube capacity.

3.11. Reagent bottles, polyethylene, 1 liter capacity.

4. Reagents

4.1. Sodium hydroxide (NaOH), anhydrous, reagent grade pellets.

4.2. Sodium chloride (NaCl), reagent grade crystals.

4.3. Calcium oxide (CaO), reagent grade powder.

4.4. Mercuric thiocyanate ($\text{Hg}(\text{SCN})_2$), reagent grade.

4.5. Ferric ammonium sulphate ($\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$), reagent grade crystals.

4.6. Nitric acid (HNO_3), concentrated reagent grade.

4.7. Acetic acid (CH_3COOH), 5% for rinsing glassware.

4.8. Calcium Oxide Solution (saturated)

Dissolve 20 g calcium oxide in distilled water. Dilute to 1 liter with distilled water and allow to stand overnight. Use supernatant liquid.

4.9. Ferric Ammonium Sulphate Solution

Dissolve 120 g ferric ammonium sulphate solution in 500 ml distilled water. Dilute to 1 liter with concentrated nitric acid. Filter if necessary and let stand for 24 hours before using.

4.10. Nitric Acid 4.4%

Add 250 ml concentrated nitric acid to 2 liters distilled water. Mix and dilute to 4 liters with distilled water.

4.11. Mercuric Thiocyanate Solution (saturated)

Add 10 g mercuric thiocyanate solution to 500 ml distilled water. Shake thoroughly and allow to stand overnight. Use the supernatant liquid.

4.12. Chloride Stock Solution (1000 mg/l Cl^-)

In a volumetric flask dissolve 1.65 g sodium chloride (oven dried and cooled in a desiccator) in distilled water and dilute to 1 liter.

4.13. Chloride Working Standards

Dilute each of the following aliquots of chloride stock solution to 500 ml in volumetric flasks: 1.0; 2.5; 5.0; 10; 20; 37.5 and 50 ml. This gives a series of working standards containing 2, 5, 10, 20, 40, 75 and 100 mg/l Cl^- respectively.

4.14. Quality Control Procedure

Quality control solutions A and B are prepared by combining sets of residues from analytical determinations in 500 ml polyethylene bottles until at least one month's supply of solution has been accumulated. Judicious blending of

samples will provide controls in the 10% to 20% and 80% to 90% range of the instrumental response without further dilutions.

These quality control solutions are run daily with the calibration standards and serve to detect anomalies in the calibration blanks, systematic errors and as a monitor of the sensitivity response of the instrumentation.

When new A and B controls are required, they should be prepared sufficiently in advance so that an overlap of at least three days of comparative data can be acquired. With vegetation samples, A and B-solutions are quite stable for at least sixty days.

5. Procedure

5.1. Sample Preparation

If a washed vegetation sample is requested, place a portion of the sample in a 12 inch porcelain dish and gently rinse for 30 seconds with a solution containing 0.05% Alcanox and 0.05% EDTA tetra sodium salt. Rinse 3 times with 1 liter distilled water and transfer wet material to a 1 liter beaker. If an unwashed sample is requested transfer a portion of sample to a new Kraft paper bag. Dry both washed and unwashed portions in a forced air oven at 80°C, grind for 48 hours in a Wiley Mill to pass through an 80 mesh screen and store in 4 oz screw cap jars.

Soil samples should be air dried and ground to pass through a 2 mm sieve.

5.2. Sample Digestion

- 5.2.1. Weigh 1.00 g dried vegetation sample or 0.2 g soil sample into a nickel crucible. (Record sample number and corresponding crucible identification number).
- 5.2.2. Add 10 ml saturated calcium oxide solution to each sample and allow to stand until the sample is thoroughly wetted. For each batch, prepare 2 blank crucibles.
- 5.2.3. Transfer samples to a drying oven and dry at 105°C until completely dry or for at least 2 hours.
- 5.2.4. Transfer dried samples to a muffle furnace at room temperature and heat to 250°C. After 90 minutes, raise temperature to 600°C and maintain this temperature for a further 2 hours. Keep furnace door closed throughout. (If fluoride is also to be measured do not allow oven temperature to rise above 525°C).
- 5.2.5. Remove crucibles from hot furnace, add 3 g sodium hydroxide pellets and return to oven with door closed for 3 minutes. Remove from oven and swirl until fused melt is partially solidified. Cool crucibles to room temperature and add 25 ml distilled water. Let stand for at least 3 hours until melt has dissolved.

NOTE: Wear face mask throughout this operation due to caustic nature of sodium hydroxide.
- 5.2.6. By repeated rinsings with distilled water transfer crucible contents to a corresponding clean, numbered 50 ml plastic volumetric flask. Adjust to a final volume of 50 ml with distilled water.

NOTE: Wear eye glasses.

5.3. Chloride Determination

REFER TO MANUFACTURER'S MANUAL FOR SET-UP, CLEANING AND CHECKING PROCEDURES FOR AUTOANALYZER SYSTEM.

- 5.3.1. Set up AutoAnalyzer as in Figure 2.
- 5.3.2. Load sampler tray with standards, A and B control solutions and prepared samples, by transferring portions of each to clean, dry tubes and inserting into alternate positions on the tray. Intermediate positions are filled with tubes of distilled water. This allows extended flushing of the system between samples and a reasonable separation of sample peaks. Sampler is run at a setting of 30 tubes per hour.

NOTE: Alternatively, sampling tray may be run at 15 tubes/hour and water-wash tubes omitted.

- 5.3.3. Prepare system for analysis by stretching and clamping proportioning tubes in manifold and insert intakes of the lines into distilled water. Switch on proportioning pump and recorder drive and let run for a few minutes.
- 5.3.4. Insert lines into the appropriate receptacles and run until the base line is established.
- 5.3.5. Switch on sampler and run the standards, blanks and samples.
- 5.3.6. During a daily run, standards will have to be run at least once more, as there is some drift with temperature changes in the system during a prolonged run. The base line should also be checked from time to time.

NOTE: Close attention must be given to samples from an area of suspected high chloride contamination since excessive chloride levels will tend to overload the system and interfere with subsequent samples. Such samples must be removed and diluted before the run is continued. Choosing the correct dilution requires experience and the operator may initially have to experiment with several before a suitable dilution is found.

NOTE: The sample sequence is always in order of appearance on the analytical working sheets, but it is convenient to identify each peak obtained on the chart as to standard, A and B and sample request number.

6. Calculation and Reporting

A calibration curve should be drawn plotting the Cl^- concentration of the standards in $\mu\text{g/ml}$ against the chart reading. For each sample and for the blank, determine Cl^- concentration corresponding to peak height.

$$\% \text{Cl}^- \text{ in sample} = (a - b) \times \frac{50}{c} \times \frac{V}{10,000}$$

Where:

a = $\mu\text{g/ml Cl}^-$ measured in solution

b = $\mu\text{g/ml Cl}^-$ measured in blank

c = sample weight

V = dilution factor

Results are reported to 0.01% Cl^- .

7. Precision and Accuracy

Within-run standard deviation based on 303 pairs of duplicate samples is 7.9%.

Calibration is controlled by 2 long-term standards (QC-A and QC-B) which are prepared by combining at least 1 month's supply of sample solution to produce a QC-A at approximately 10% and a QC-B at approximately 80% of scale. The quality control solutions must not vary by more than 2 standard deviations from their respective long-term means.

8. Bibliography

- 8.1. Chapman, H.D. (1965). Diagnostic Criteria for Plants and Soils. Department of Soils and Plant Nutrition, University of California, California. 793 p.

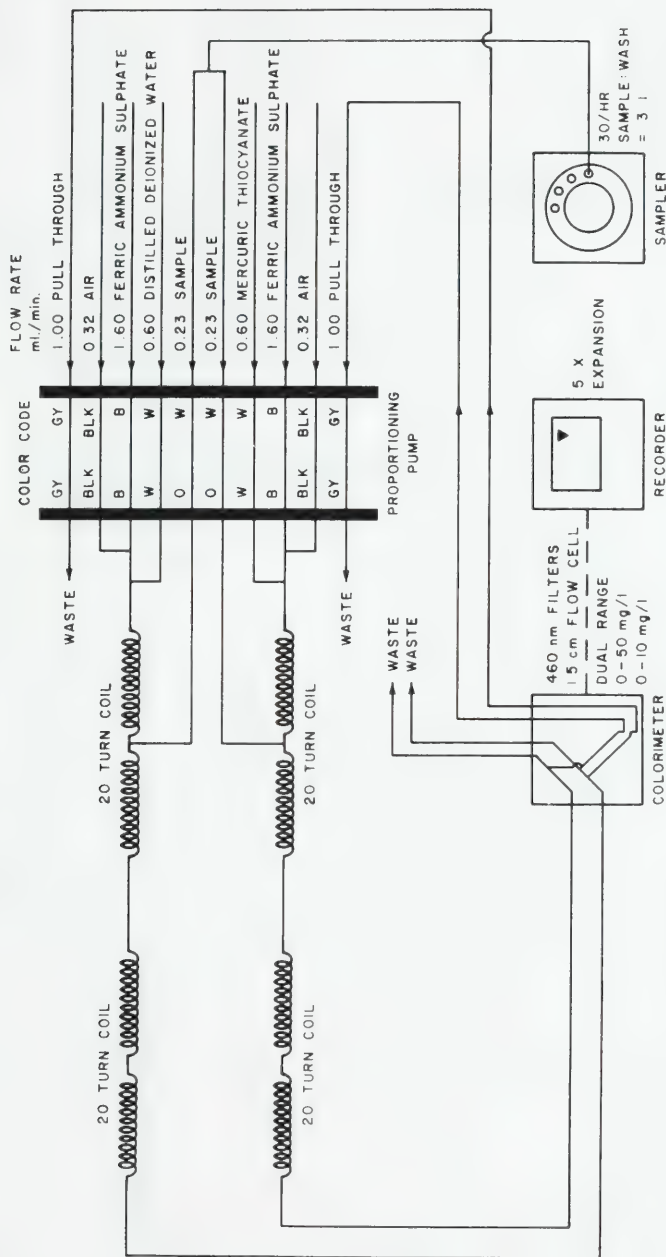


FIGURE 1 — AUTOANALYZER AAI SYSTEM FOR CHLORIDE DETERMINATION ON SURFACE WATER SAMPLES

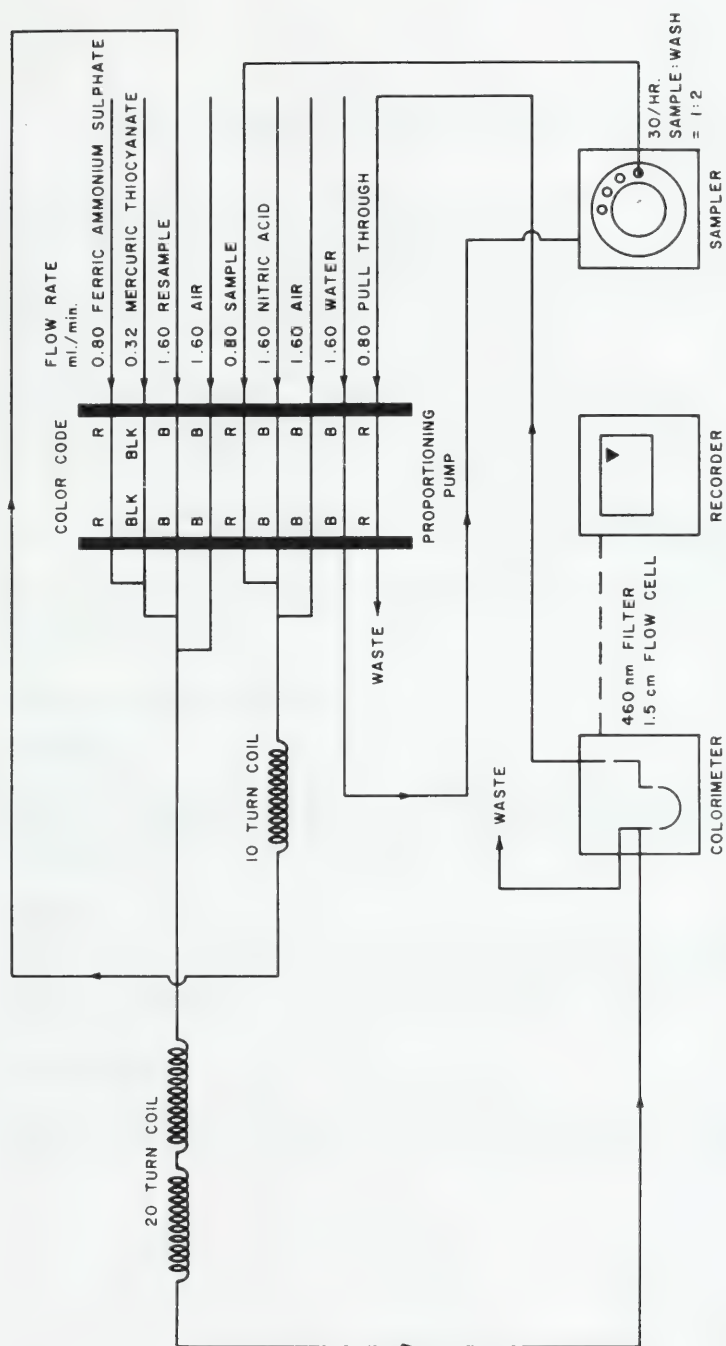


FIGURE 2 — AUTOANALYZER AAI SYSTEM FOR CHLORIDE DETERMINATIONS IN
SOIL AND VEGETATION SAMPLES

THE DETERMINATION OF CHLORINATED DIBENZO-P-DIOXINS

Except for scientific research purposes, polychlorinated dioxins (PCDD) are not intentionally manufactured, nor do they occur naturally. Dioxins occur exclusively as accidental or unwanted by-products in the manufacture of other chemicals such as that of the herbicide 2,4,5-T. PCDD's can also be formed and discharged during incineration of municipal and chemical wastes. Chemicals such as chlorobenzenes and PCB's have been shown to form PCDD's during incineration, and the combustion, in the presence of chlorine, of many other organics can result in PCDD formation.

Although one member, 2,3,7,8-TCDD, (TCDD) of the dioxin family has been labelled the most toxic poison known to man, exposure of the general populace to this deadly compound is very restricted. Exposure to the 74 other, less toxic, chlorinated isomers of dibenzo-p-dioxin may be more widespread. However, the effects of long-term human exposure to low levels of these dioxins are not well documented.

The effects on test animals from chronic exposure to TCDD include: chloracne, degenerative liver changes, teratogenic, fetotoxic and carcinogenic effects.

Although the minimum lethal dose of TCDD is 1.0×10^{-6} g/kg, exposure to such a dose would be limited to industrial/occupational accidents or accidental consumption of a contaminated product.

Sample Handling and Preservation

Water Samples

Samples must be collected in glass bottles with foil or Teflon lined screw-caps. Use 1 liter, amber bottles that are solvent rinsed and labelled "For Pesticide and PCB Analysis Only". A minimum sample size of 1 liter is required but duplicate 1 liter samples should be submitted.

Fish Samples

Whole fish must be collected and delivered in solvent-rinsed foil; samples must be frozen immediately after collection. Samples must not come in contact with plastic materials whatsoever. Samples must remain frozen. Fish that have been thawed will not be analyzed.

Selection of method

Method A is a GC/MS method and is used for the analysis of total TCDD's in water. Method B is a GC/MS method which is used for the determination of 2, 3, 7, 8-TCDD in fish.

CHLORINATED DIBENZO-P-DIOXINS

GC/MS Method A

SUMMARY

Matrix.	Water.
Substances determined.	Total TCDD in water.
Interpretation of results.	Results are reported in ng/l.
Principle of method.	Sample is extracted with solvent, and concentrated, then cleaned-up to remove interferences. The final extract is analyzed by gas chromatography and residues are confirmed by GC/MS. Quantitation is done by peak area comparison and mass number.
Time required for analysis.	Under optimum conditions 15 water samples may be analyzed in 5 days.
Range of application.	From 0.25 ng/l for water.
Standard deviation.	Not available.
Accuracy.	94% recovery from water fortified with 1 ng/l of TCDD.
Detection criteria.	0.25 ng/l total TCDD
Interferences and shortcomings.	The EC detector is not specific and many naturally occurring materials will interfere which may not be completely removed by the exhaustive clean-up procedure used. MS confirmation removes most other doubts about dioxin identity.
Minimum volume of sample.	Duplicate 1 liter samples.
Preservation and sample container.	Only 1 liter solvent-rinsed amber glass bottles are acceptable. Screw-caps must be foil or Teflon lined to prevent contamination. No preservatives are required, but samples must be refrigerated at 4°C.

**Safety
considerations.**

Due to the high toxicity of PCDD's, special, stringent precautions must be taken to ensure a safe operation.

All air exhausted from the laboratory must be passed through specially designed fumehoods containing carbon and hepa filters in order to prevent atmospheric contamination.

Disposable gloves must always be worn when handling equipment, glassware, samples or solutions suspected of containing dioxins. Back-tied lab coats should be worn and special cleaning procedures should be instituted for them.

A special disposal protocol should be established for gloves and other disposable items.

Glassware must be carefully washed, scrubbed and rinsed before use. The last solvent rinse is collected, concentrated and analyzed by GC/ECD to ensure that no trace contaminants remain. All glassware rinsing should be done in a special fumehood.

Extreme care must be taken to prevent contact of gloves with ground-glass joints and inner parts of glassware, in order to avoid contamination with phthalates which interfere with the TCDD determination.

CHLORINATED DIOXINS

GC/MS Method. A: Water

1. Introduction

Dioxins are extracted from water with organic solvent. The extract is evaporated and cleaned-up if necessary by column chromatography. This extract is concentrated, screened by capillary gas chromatography and the presence of dioxin is confirmed by GC/MS.

2. Interferences and Shortcomings

Many naturally occurring substances interfere with the EC determination of chlorinated dioxins, especially at the ultra-trace levels determined. Most of these are eliminated by the column clean-up procedure used. MS analysis at given mass numbers removes most other doubts about the identity of PCDD residues.

One source of contamination can be eliminated by thorough multi-phase clean-up of all glassware, using ultra-pure solvents and taking extreme care that protective gloves never come into contact with ground-glass parts.

3. Apparatus

ALL GLASSWARE MUST BE RINSED THOROUGHLY WITH SOLVENT PRIOR TO USE.

3.1. Extraction Procedure - Water

- 3.1.1. Magnetic stirrer and bar.
- 3.1.2. Pasteur pipette, glass, disposable.
- 3.1.3. Volumetric flask, Pyrex, 1000 ml, with glass stopper.
- 3.1.4. Blow down apparatus.
- 3.1.5. Graduated cylinder, Pyrex, 1000 ml.
- 3.1.6. Centrifuge tube, Pyrex, 50 ml.
- 3.1.7. Reacti-vial, Pyrex, 0.10 ml, with screw cap and septum.

3.2. Chromatographic Packings - Preparation

- 3.2.1. Silicic Acid
 - 3.2.1.1. Tube furnace, 500°C maximum.
 - 3.2.1.2. Pyrex tube, 45 cm x 2.5 cm I.D.
 - 3.2.1.3. Bottle, Pyrex, 250 ml.
 - 3.2.1.4. Desiccator

3.2.2. Silver nitrate packing

- 3.2.2.1. Balance, top-loading, to 0.01 grams.
- 3.2.2.2. Graduated cylinder, Pyrex, 25 ml.
- 3.2.2.3. Erlenmeyer flask, 125 ml, with glass stopper.
- 3.2.2.4. Pyrex tube, 45 cm x 2.5 cm I.D.
- 3.2.2.5. Tube furnace, 500°C maximum.
- 3.2.2.6. Glass bottle, amber, solvent-rinsed.
- 3.2.2.7. Desiccator.

3.2.3. Sodium hydroxide/silicic acid packing

- 3.2.3.1. Balance, top-loading, to 0.01 grams.
- 3.2.3.2. Erlenmeyer flasks, 1000 ml, with Teflon lined screw cap, and 250 ml, with glass stopper.

3.2.4. Sulphuric acid/silicic acid packing

- 3.2.4.1. Balance, top-loading, to 0.01 g.
- 3.2.4.2. Graduated cylinders, Pyrex, 50 ml and 25 ml.
- 3.2.4.3. Erlenmeyer flask, Pyrex, 250 ml, with glass stopper.

3.2.5. Alumina packing

- 3.2.5.1. Tube furnace, 500°C maximum.
- 3.2.5.2. Pyrex tube, 45 cm x 2.5 cm I.D.
- 3.2.5.3. Desiccator.
- 3.2.5.4. Bottle, Pyrex.

3.3. Clean-up

- 3.3.1. Top column: Pyrex tubing, 10 cm x 10 mm I.D., with an inner 12/30 glass joint at the bottom and an outer 12/30 glass joint at the top, into which fits a 100 ml reservoir.
- 3.3.2. Alumina column: Pyrex tubing, 28 cm x 6 mm I.D., with Teflon stopcock at bottom and outer 12/30 glass joint at the top, into which fits a 100 ml reservoir.
- 3.3.3. Pasteur pipettes, glass, disposable.
- 3.3.4. Centrifuge tubes, Pyrex, 50 ml, with glass stopper.
- 3.3.5. Reacti-vial, 0.10 ml, glass, with screw cap and septum.

3.4. Screening/Confirmation

3.4.1. GC screening

- 3.4.1.1. Dual electron capture detector (ECD), dual capillary gas chromatograph: Hewlett Packard 5880 or equivalent.

3.4.1.2. Ni⁶³ electron capture detectors.

3.4.1.3. 30 m SE30 fused silica capillary column.

3.4.1.4. 25 m SE54 fused silica capillary column.

3.4.1.5. Operating conditions:

Hydrogen: 1.2 ml/min
Argon/methane (95%): 30 ml/min
Injection port: 300°C
Injection: 76°C
Initial hold: 1.0 minute
Temperature program:
76 to 175°C at 25°C/min
175 to 275°C at 2°C/min
Final hold: 275°C for 10 minutes

3.4.2. GC/MS confirmation

3.4.2.1. Finnigan 4500 GC/MS equipped with Grob-type capillary injector, or equivalent.

3.4.2.2. 30 m DB-1 fused silica capillary column.

3.4.2.3. GC Operating conditions:

Injection port: 270°C
Injection: 76°C
Initial hold: 2.0 minute
Ramp rate: 20°C/min to 230°C.
Temperature program:
230°C to 300°C at 5°C/min
Final hold: 300°C for 20 minutes.

3.4.2.4. MS operating conditions.

EM: 1250 - 2000 volts
Electron energy: 32v
SIM window size: 0.5 amu
Ion masses: 319.9 321.9 323.9 335.9
Dwell times (μsec): 210 210 210 210

4. Reagents

4.1. Extraction Procedure

- 4.1.1. Pentane, distilled in glass, residue-free.
- 4.1.2. Nitrogen, femtgrade (4.5).
- 4.1.3. Sodium sulphate (Na_2SO_4), anhydrous, granular
- 4.1.4. Glass wool, solvent-rinsed.

4.2. Chromatographic packings - Preparation

4.2.1. Silicic Acid Packing

- 4.2.1.1. Silicic acid, chromatographic grade, 100/200 mesh, Bio-Sil A.
- 4.2.1.2. Nitrogen, femtgrade (4.5).
- 4.2.1.3. Methanol (CH_3OH), HPLC grade.
- 4.2.1.4. Dichloromethane (CH_2Cl_2), distilled in glass, residue free.
- 4.2.1.5. Glass wool, solvent-rinsed.
- 4.2.1.6. Silica-gel desiccant.

4.2.2. Silver nitrate packing

- 4.2.2.1. AgNO_3 , reagent grade.
- 4.2.2.2. Water, deionized.
- 4.2.2.3. Silicic acid, activated (5.2.1).
- 4.2.2.4. Glass wool, solvent-rinsed.
- 4.2.2.5. Nitrogen, femtgrade (4.5).
- 4.2.2.6. Silica-gel desiccant.

4.2.3. Sodium hydroxide/silicic acid packing

- 4.2.3.1. NaOH , pellets, reagent grade.
- 4.2.3.2. Water, deionized.
- 4.2.3.3. Silicic acid, activated (5.2.1.).

4.2.4. Sulphuric acid/silicic acid packing

- 4.2.4.1. H_2SO_4 , concentrated
- 4.2.4.2. Silicic acid, activated (5.2.1.).

4.2.5. Alumina packing

- 4.2.5.1. Aluminium oxide, chromatographic grade, 100/200 mesh, basic alumina, Bio-Rad AG-10.
- 4.2.5.2. Glass wool, solvent-rinsed.
- 4.2.5.3. Nitrogen, femtgrade (4.5).
- 4.2.5.4. Dichloromethane (CH_2Cl_2), distilled in glass, residue free.

4.2.5.5. Silica-gel desiccant.

4.3. **Clean-up water**

- 4.3.1. Alumina (5.2.5.).
- 4.3.2. Glass-wool, solvent rinsed.
- 4.3.3. Hexane ($\text{CH}_3(\text{CH}_2)_4\text{CH}_3$), HPLC grade.
- 4.3.4. Carbon tetrachloride (CCl_4), distilled in glass, residue free.
- 4.3.5. Isooctane ($(\text{CH}_3)_3\text{CCH}_2\text{CH}(\text{CH}_3)_2$), distilled in glass, residue-free.

4.4. **Screening/confirmation**

4.4.1. GC screening

- 4.4.1.1. Gases:
 - Argon/methane (95%): pre-purified.
 - Hydrogen: pre-purified.

4.4.2. GC/MS confirmation

- 4.4.2.1. Gases:
 - Argon/methane (95%): pre-purified.
 - Helium: pre-purified.
- 4.4.2.2. Stock TCDD solution. Dissolve 10 μg of TCDD in 100 μl of anisole.
- 4.4.2.3. TCDD standard (33 $\text{pg}/\mu\text{l}$). Dilute the stock solution with isooctane to make a 33 $\text{pg}/\mu\text{l}$ solution.
- 4.4.2.4. Hexane ($\text{CH}_3(\text{CH}_2)_4\text{CH}_3$), HPLC grade.

4.5. **Femtogas purification system**

Pack a 30 cm x 2 cm I.D. Pyrex tube as follows:

- Alternate 6 cm layers of the following adsorbents with 1 cm layers of Ottawa sand beginning with the sand:
 - 2% Apiezon L + 2% graphite, (Carbopack B) on 60/80 Chromosorb W-AW.
 - molecular sieve, 80/100 mesh (activated at 400°C with nitrogen purge at 100 ml/min for 4 hours).
 - 20% H_2SO_4 /silicic acid.
 - Carbosieve S, 60/80 mesh.

The tube is plugged at each end with SS mesh and glass wool.

Connect this tube into the pre-purified nitrogen line, using only SS tubing, due to the corrosive properties of the H_2SO_4 packing.

The resulting gas is called femtograde nitrogen throughout this methodology.

5. **Procedure**

5.1. **Extraction - Water**

- 5.1.1. Mark sample level on bottle for volume measurement, using a 1000 ml graduated cylinder, following extraction.

- 5.1.2. Transfer sample to 1000 ml volumetric flask, containing magnetic bar.
- 5.1.3. Rinse sample bottle with 15 ml of pentane.
- 5.1.4. Transfer pentane rinse to sample in volumetric flask and stopper.
- 5.1.5. Add 100 mls of pentane.
- 5.1.6. Stir for an hour, ensuring that vortex reaches bottom of flask.
- 5.1.7. Remove, pentane with pasteur pipette into round bottom flask.
- 5.1.8. Re-extract sample two additional times by stirring for 1 hour with 100 ml of pentane.
- 5.1.9. After the third pentane extraction, distilled, deionized water is added to the flask to raise the organic layer to the narrow neck of the flask to facilitate solvent removal.
- 5.1.10. Rotary-evaporate combined dry extracts to 1 ml.
- 5.1.11. Further concentrate extract by transferring to a 0.1 ml Reacti-vial, drop-wise and evaporate under a stream of femtograde nitrogen. Alternate drop-wise addition and evaporation until whole extract has been transferred.
- 5.1.12. Rinse centrifuge tube with 1 ml of pentane and evaporate as above, to dryness.
- 5.1.13. Make up residue to 10 μ l with isooctane for GC/MS analysis.

NOTE: To ensure staff safety, extreme care must be taken in all transfer and evaporation steps, especially when handling concentrated solutions, which might contain TCDD.

5.2. Chromatographic packing - Preparation

- 5.2.1. **Silicic acid**
 - 5.2.1.1. Silicic acid is dried in a glass tube (3.2.1.2.) at 180°C for 30 minutes under a continuous purge of femtograde nitrogen.
 - 5.2.1.2. Continue nitrogen purge during 15 minute cool down period.
 - 5.2.1.3. Sequentially elute silicic acid with 150 ml portions of methanol and dichloromethane.
 - 5.2.1.4. After all dichloromethane has eluted, return tube containing silicic acid to furnace set at 50°C and purge with femtograde nitrogen.
 - 5.2.1.5. Over a period of 30 minutes, increase temperature to 180°C, venting gaseous effluent to a fumehood.

- 5.2.1.6. Further activate the packing at 180°C for 90 minutes, always purging with nitrogen.
- 5.2.1.7. Continue nitrogen purge during cool-down period.
- 5.2.1.8. Transfer activated silicic acid to a dry, solvent-rinsed bottle for storage in a desiccator, over silica-gel.

5.2.2. **Silver nitrate packing**

A 10% AgNO₃ on silicic acid packing is prepared as follows:

- 5.2.2.1. Weigh 6.0 g of AgNO₃.
- 5.2.2.2. Dissolve AgNO₃ in 23 ml of deionized water.
- 5.2.2.3. Weigh 53.0 g of activated silicic acid (5.2.1.) into a 125 ml Erlenmeyer flask.
- 5.2.2.4. Add dissolved AgNO₃ to flask containing silicic acid and shake by hand until powder is free-flowing.
- 5.2.2.5. Allow packing to stand for 30 minutes at room temperature.
- 5.2.2.6. Transfer packing to glass tube (3.2.1.2.) plugged with glass wool and place in tube furnace at 70°C under a continuous flow of femtgrade nitrogen.
- 5.2.2.7. Over a 5-hour period, increase temperature, stepwise, to 120°C.
- 5.2.2.8. After condensation of deionized water has ceased, activate packing at 125°C for 15 hours. Continue nitrogen purge during cool-down period.
- 5.2.2.9. Transfer cool packing to an amber glass bottle and store in a desiccator, over silica-gel.

5.2.3. **Sodium hydroxide/silicic acid**

A 33% NaOH/silicic acid packing is prepared as follows:

- 5.2.3.1. Weigh 40.0 g of NaOH pellets.
- 5.2.3.2. Dissolve NaOH in 1000 ml of deionized water in a screw-capped Erlenmeyer flask. This gives a 1N NaOH solution.
- 5.2.3.3. Weigh 67.0 g of the activated silicic acid (5.2.1.) in a 250 ml Erlenmeyer flask.
- 5.2.3.4. Weigh 33.0 g of the 1N NaOH and add, slowly, to the flask containing the silicic acid. Shake vigorously until powder is free-flowing.
- 5.2.3.5. Store in desiccator, over silica gel.

5.2.4. Sulphuric acid/silicic acid packing

5.2.4.1. Weigh 42.0 g of activated silicic acid (5.2.1.) into a 250 ml Erlenmeyer flask.

5.2.4.2. Measure 33 ml of concentrated H_2SO_4 .

5.2.4.3. Carefully add the H_2SO_4 to the silicic acid in the flask and stopper.

5.2.4.4. Shake vigorously by hand until powder is free-flowing.

The above packing (42 g of silicic acid/33 ml of H_2SO_4) contains 44% H_2SO_4 .

For 22% H_2SO_4 /silicic acid use 58.5 g of silicic acid and 16.5 ml of H_2SO_4 .

NOTE: After treatment with H_2SO_4 , the silicic acid retains the corrosive properties of the acid and should be handled with great care.

5.2.5. Alumina packing

5.2.5.1. Pour alumina (4.2.5.) into the glass-wool plugged tube (3.2.1.2.) and dry in tube furnace at 300°C for 60 minutes under a continuous purge of femtograd nitrogen.

5.2.5.2. Cool to room temperature continuing nitrogen purge.

5.2.5.3. Elute 150 ml of dichloromethane through the packing.

5.2.5.4. Return tube to furnace, re-establish femtograd nitrogen purge.

5.2.5.5. Slowly increase temperature to 180°C , over a period of 30 minutes and maintain until all dichloromethane has vented.

5.2.5.6. Increase temperature slowly to 300°C and maintain for 90 minutes.

5.2.5.7. Cool packing under continuous nitrogen purge.

5.2.5.8. Transfer packing to glass bottle and store in desiccator over silica-gel.

5.3. Clean-up

In most cases of raw and drinking water, this step is unnecessary, however, if interfering contaminants are present or suspected the following procedure should be followed.

5.3.1. Load top column (3.3.1.) (plugged with glass wool) sequentially with the following packings:

1.0 g of AgNO₃/silicic acid (5.2.2.).
0.5 g of silicic acid (5.2.1.).
1.0 g of 33% NaOH/silicic acid (5.2.3.).
0.5 g silicic acid.
2.0 g of 44% H₂SO₄/silicic acid (5.2.4.).

- 5.3.2. Load alumina column (3.3.2.) plugged with glass wool, with basic alumina (5.2.5.) to a height of 21 cm.
- 5.3.3. Fit packed alumina column into bottom of top column.
- 5.3.4. Apply sample extract (5.1.11) to top column using a disposable pipette.
- 5.3.5. Rinse sample container with 2 x 1 ml of hexane and transfer to column.
- 5.3.6. Fit reservoir into top column and elute with 100 ml of hexane as soon as sample has reached top of packing.
- 5.3.7. Discard top column and fit clean reservoir into top of alumina column.
- 5.3.8. Elute remaining alumina column sequentially with:
 - a) 40 ml of 10% CCl₄/hexane.
 - b) 10 ml of hexane.
 - c) 30 ml of dichloromethane, collected in a 50 ml centrifuge tube.
- 5.3.9. Carefully blow-down fraction c) containing the TCDD's, under a stream of femtograde nitrogen, to dryness.
- 5.3.10. Make-up residue in 1 ml of hexane and transfer dropwise to a 0.10 ml Reacti-vial, previously rinsed with solvent.
- 5.3.11. Gently evaporate solvent under a stream of femtograde nitrogen.
- 5.3.12. Continue dropwise addition and evaporation until all hexane has been transferred.
- 5.3.13. Rinse centrifuge tube with 1 ml of hexane and evaporate to dryness as described above.
- 5.3.14. Make up to 10 µl with isoctane.

5.4. Screening/confirmation

The final identification/quantitation step involves detection of 2,3,7,8-TCDD by monitoring ions at m/z 319.9, 321.9 and 323.9 at the correct retention time. The retention time is established daily by calibration using TCDD standards ranging from 6 to 60 pg. Sensitivity is 10 pg under normal operating conditions (3.4.1. and 3.4.2.). TCDD is reported as a total TCDD (all isomers) measured against a 2,3,7,8-TCDD standard.

5.4.1. GC/screening

- chromatographic peak must elute within ± 0.02 minute of the standard retention time.
- integrated peak areas from each capillary column must agree within $\pm 20\%$.

Samples meeting both these criteria are further analyzed by mass spectrometry.

5.4.2. GC/MS Confirmation

Samples must meet the following criteria for confirmation as 2,3,7,8-TCDD:

1. Correct capillary column (ECD and/or GC/MS) retention time of 2,3,7,8-TCDD.
2. Correct chlorine isotope ratios of m/z 319.9/321.9/323.9 within 15% of theoretical ratios of 76:100:48.
3. Response of m/e 319.9 and 321.9 must be greater than 2.5 times the noise level.

Samples are quantitated against 2,3,7,8-TCDD standard, but reported as total TCDD.

6. Calculation and Reporting

Results are obtained from integration of peak areas and comparison to a standard solution (4.4.2.3.).

7. Precision and Accuracy

The recovery of a water sample spiked with 1 ng/l TCDD was 94%.

8. Bibliography

None.

CHLORINATED DIBENZO-P-DIOXINS

GC/MS Method B

SUMMARY

Matrix.	Fish.
Substances determined.	2,3,7,8-TCDD in fish.
Interpretation of results.	Results are reported in pg/g.
Principle of method.	Sample is extracted with solvent, and concentrated, then cleaned-up to remove interferences. The final extract is analyzed by gas chromatography and residues are confirmed by GC/MS. Quantitation is done by peak area comparison and mass number.
Time required for analysis.	Under optimum conditions 20 fish samples can be analyzed per week.
Range of application.	From 5 pg/g for fish.
Standard deviation.	Not available.
Accuracy.	83% recovery from fish fortified with 100 pg/g of 2,3,7,8-TCDD.
Detection criteria.	5 pg/g 2,3,7,8-TCDD.
Interferences and shortcomings.	The EC detector is not specific and many naturally occurring materials will interfere which may not be completely removed by the exhaustive clean-up procedure used. MS confirmation removes most other doubts about dioxin identity.
Minimum volume of sample.	Whole fish.
Preservation and sample container.	Samples must be frozen immediately after collection and kept frozen thereafter. Samples must be wrapped in solvent-rinsed foil only.
Safety considerations.	<p>Due to the high toxicity of PCDD's, special, stringent precautions must be taken to ensure a safe operation.</p> <p>All air exhausted from the laboratory must be passed through specially designed fumehoods containing carbon and hepa filters in order to prevent atmospheric contamination.</p>

Disposable gloves must always be worn when handling equipment, glassware, samples or solutions suspected of containing dioxins. Back-tied lab coats should be worn and special cleaning procedures should be instituted for them.

A special disposal protocol should be established for gloves and other disposable items.

Glassware must be carefully washed, scrubbed and rinsed before use. The last solvent rinse is collected, concentrated and analyzed by GC/ECD to ensure that no trace contaminants remain. All glassware rinsing should be done in a special fumehood.

Extreme care must be taken to prevent contact of gloves with ground-glass joints and inner parts of glassware, in order to avoid contamination with phthalates which interfere with the TCDD determination.

CHLORINATED DIOXINS

GC/MS Method B: Fish

1. Introduction

Fish homogenates are digested using concentrated HCl then the dioxins are extracted with organic solvent. The extract is evaporated and exhaustively cleaned-up using two dual-column chromatography systems followed by reverse phase HPLC. This resulting extract is concentrated, screened by capillary gas chromatography and the presence of dioxin is confirmed by GC/MS.

2. Interferences and Shortcomings

Many naturally occurring substances interfere with the EC determination of chlorinated dioxins, especially at the ultra-trace levels determined. Most of these are eliminated by the exhaustive column clean-up procedure used. MS analysis at given mass numbers removes most other doubts about the identity of TCDD residues.

One source of contamination can be eliminated by thorough multi-phase clean-up of all glassware, using ultra-pure solvents and taking extreme care that protective gloves never come into contact with ground-glass parts.

3. Apparatus

ALL GLASSWARE MUST BE RINSED THOROUGHLY WITH SOLVENT PRIOR TO USE.

3.1. Extraction Procedure - Fish

- 3.1.1. Virtis homogenizer.
- 3.1.2. Erlenmeyer flask, Pyrex, 250 ml, with Teflon screw cap.
- 3.1.3. Flask, Pyrex, 250 ml, round-bottom with 24/40 glass joint.
- 3.1.4. Measuring cylinders, Pyrex, graduated, 100 ml, 50 ml.
- 3.1.5. Wrist-action shaker.
- 3.1.6. Pipettes, glass, disposable.
- 3.1.7. Rotary evaporator.

3.2. Chromatographic Packings - Preparation

- 3.2.1. Silicic Acid
 - 3.2.1.1. Tube furnace, 500°C maximum.
 - 3.2.1.2. Pyrex tube, 45 cm x 2.5 cm I.D.
 - 3.2.1.3. Bottle, Pyrex, 250 ml.
 - 3.2.1.4. Desiccator

3.2.2. Silver nitrate packing

- 3.2.2.1. Balance, top-loading, to 0.01 grams.
- 3.2.2.2. Graduated cylinder, Pyrex, 25 ml.
- 3.2.2.3. Erlenmeyer flask, 125 ml, with glass stopper.
- 3.2.2.4. Glass tube, 45 cm x 2.5 cm I.D.
- 3.2.2.5. Tube furnace, 500°C maximum.
- 3.2.2.6. Glass bottle, amber, solvent-rinsed.
- 3.2.2.7. Desiccator.

3.2.3. Sodium hydroxide/silicic acid packing

- 3.2.3.1. Balance, top-loading, to 0.01 grams.
- 3.2.3.2. Erlenmeyer flask, 1000 ml, with Teflon lined screw cap, and 250 ml, with glass stopper.

3.2.4. Sulphuric acid/silicic acid packing

- 3.2.4.1. Balance, top-loading, to 0.01 g.
- 3.2.4.2. Graduated cylinders, Pyrex, 50 ml and 25 ml.
- 3.2.4.3. Erlenmeyer flask, Pyrex, 250 ml, with glass stopper.

3.2.5. Alumina packing

- 3.2.5.1. Tube furnace, 500°C maximum.
- 3.2.5.2. Pyrex tube, 45 cm x 2.5 cm I.D.
- 3.2.5.3. Desiccator.
- 3.2.5.4. Bottle, Pyrex.

3.3. Clean-up - Fish

- 3.3.1. H₂SO₄ column: Pyrex tubing, 10 cm x 10 mm I.D., with an inner 12/30 glass joint at the bottom and an 12/30 outer glass joint at the top into which fits a 100 ml reservoir.
- 3.3.2. Alumina column: Pyrex tubing, 28 cm x 6 mm I.D., with Teflon stopcock at bottom and 12/30 outer glass joint at top.
- 3.3.3. Reservoir, Pyrex, 100 ml, with 12/30 inner joint.
- 3.3.4. Pasteur pipettes, glass, disposable.
- 3.3.5. Centrifuge tubes, Pyrex, 50 ml, with glass stopper.
- 3.3.6. Reacti-vial, Pyrex, 0.10 ml, with screw cap and septum.
- 3.3.7. Blow-down apparatus.

- 3.3.8. AgNO_3 column, Pyrex tubing, 10 cm x 6 mm I.D., with an 12/30 outer glass joint at the top into which fits a 100 ml reservoir, and with a Teflon stopcock and a 12/30 inner glass joint as the bottom.
- 3.3.9. HPLC instrumentation: Waters, model 6000A solvent delivery system, or equivalent, with a U.V. Spectrophotometer, set at 254 nm, and a Rheodyne U6K injection system, or equivalent, and with two reverse phase columns connected in series.
- 3.3.10. Syringe, Pyrex, with SS or Teflon parts, 100 μl , for HPLC (must be correct fit for injector).
- 3.3.11. Syringe, 50 μl , Pyrex with SS or Teflon parts.
- 3.3.12. Volumetric flask, Pyrex, 10 ml, with glass stopper.
- 3.3.13. Graduated cylinders, Pyrex, 100 ml, 50 ml, 25 ml and 10 ml.

3.4. Screening/confirmation

3.4.1. GC screening

- 3.4.1.1. Dual electron capture detector (ECD), dual capillary gas chromatograph: Hewlett Packard 5880 or equivalent.
- 3.4.1.2. Ni^{63} electron capture detectors.
- 3.4.1.3. 25 m SP2100 fused silica capillary column.
- 3.4.1.4. 25 m SE54 fused silica capillary column.
- 3.4.1.5. Operating conditions:

Hydrogen:	1.2 ml/min
Argon/methane (95%):	30 ml/min
Injection port:	300°C
Detector:	310°C
Injection:	76°C
Initial hold:	1.0 minute
Temperature program:	76 to 175°C at 25°C/min
	175 to 275°C at 2°C/min
Final hold:	10 minutes

3.4.2. GC/MS confirmation

- 3.4.2.1. Finnigan 4023 GC/MS equipped with a Grob-type capillary injector, or equivalent.
- 3.4.2.2. 30 m DB-1 fused silica capillary column.
- 3.4.2.3. GC Operating conditions:

Injection port:	270°C
Injection:	76°C
Initial hold:	2.0 minute
Ramp rate:	20°C/min to 230°C.
Temperature program:	230°C to 300°C at 5°C/min
Final hold:	20 minutes.

3.4.2.4. MS operating conditions.

EM:	1250 - 2000volts			
Electron energy:	30v			
SIM window size:	0.5 amu			
Ion masses:	319.9	321.9	323.9	335.9
Dwell times (μ sec):	210	210	210	210

4. Reagents

4.1. Extraction Procedure - Fish

- 4.1.1. Hydrochloric acid (HCl).
- 4.1.2. Hexane ($\text{CH}_3(\text{CH}_2)_4\text{CH}_3$), HPLC grade.
- 4.1.3. C^{13} 2,3,7,8-TCDD internal standard in hexane at a concentration of 1 ng/ml.

4.2. Chromatographic packings - Preparation

4.2.1. Silicic Acid Packing

- 4.2.1.1. Silicic acid, chromatographic grade, 100/200 mesh, Bio-Sil A.
- 4.2.1.2. Nitrogen, femtograde (4.5).
- 4.2.1.3. Methanol (CH_3OH), HPLC grade.
- 4.2.1.4. Dichloromethane (CH_2Cl_2), distilled in glass, residue free.
- 4.2.1.5. Glass wool, solvent-rinsed.
- 4.2.1.6. Silica-gel desiccant.

4.2.2. Silver nitrate packing

- 4.2.2.1. AgNO_3 , reagent grade.
- 4.2.2.2. Water, deionized.
- 4.2.2.3. Silicic acid, activated (5.2.1).
- 4.2.2.4. Glass wool, solvent-rinsed.
- 4.2.2.5. Nitrogen, femtograde (4.5).
- 4.2.2.6. Silica-gel desiccant.

4.2.3. Sodium hydroxide/silicic acid packing

- 4.2.3.1. NaOH, pellets, reagent grade.
- 4.2.3.2. Water, deionized.
- 4.2.3.3. Silicic acid, activated (5.2.1).

4.2.4. Sulphuric acid/silicic acid packing

- 4.2.4.1. H_2SO_4 , concentrated
- 4.2.4.2. Silicic acid, activated (5.2.1).

4.2.5. **Alumina packing**

- 4.2.5.1. Aluminium oxide, chromatographic grade, 100/200 mesh, basic alumina, Bio-Rad AG-10.
- 4.2.5.2. Glass wool, solvent-rinsed.
- 4.2.5.3. Nitrogen, femtgrade (4.5).
- 4.2.5.4. Dichloromethane (CH_2Cl_2), distilled in glass, residue free.
- 4.2.5.5. Silica-gel desiccant.

4.3. **Clean-up - Fish**

- 4.3.1. 44% H_2SO_4 /silicic acid (5.2.3.).
- 4.3.2. 22% H_2SO_4 /silicic acid (5.2.3.).
- 4.3.3. Basic alumina (5.2.4.).
- 4.3.4. Hexane ($\text{CH}_3(\text{CH}_2)_4\text{CH}_3$), HPLC grade.
- 4.3.5. Dichloromethane (CH_2Cl_2), distilled in glass, residue-free.
- 4.3.6. Nitrogen, femtgrade (4.5.).
- 4.3.7. AgNO_3 /silicic acid (5.2.2.).
- 4.3.8. Carbon tetrachloride (CCl_4), distilled in glass, residue-free.
- 4.3.9. Methanol (CH_3OH), HPLC grade.
- 4.3.10. HPLC columns, 2 Brownlee, Spheri 5, RP18, or equivalent, connected in series.
- 4.3.11. HPLC solvent, 100% methanol, at 1 ml/minute.
- 4.3.12. Sodium bicarbonate, 1% NaHCO_3 1%. Dissolve 10 g of sodium bicarbonate in litre of deionized water.
- 4.3.13. Isooctane ($(\text{CH}_3)_3\text{CCH}_2\text{CH}(\text{CH}_3)_2$), distilled in glass, residue-free.

4.4. **GC/MS Analysis**

4.4.1. GC identification

- 4.4.1.1. Gases:
Argon/methane (95%): pre-purified.
Hydrogen: pre-purified.

4.4.2. MS confirmation

- 4.4.2.1. Gases:
Argon/methane (95%): pre-purified.
Hydrogen: pre-purified.
- 4.4.2.2. Stock TCDD solution. Dissolve 10 μg of TCDD in 100 μl of anisole.
- 4.4.2.3. TCDD standard (33 $\text{pg}/\mu\text{l}$). Dilute the stock solution with isooctane to make a 33 $\text{pg}/\mu\text{l}$ solution.
- 4.4.2.4. C^{13} 2,3,7,8-TCDD at a concentration of 1 ng/ml in hexane.
- 4.4.2.5. Hexane ($\text{CH}_3(\text{CH}_2)_4\text{CH}_3$), HPLC grade.

4.5. Femtogas purification system

Pack a 30 cm x 2 cm I.D. Pyrex tube as follows:

Alternate 6 cm layers of the following adsorbents with 1 cm layers of Ottawa sand beginning with the sand:

- 2% Apiezon L + 2% graphite, (Carbopack B) on 60/80 Chromosorb W-AW.
- molecular sieve, 80/100 mesh (activated at 400°C with nitrogen purge at 100 ml/min for 4 hours).
- 20% H₂SO₄/silicic acid.
- Carbosieve S, 60/80 mesh.

The tube is plugged at each end with SS mesh and glass wool.

Connect this tube into the pre-purified nitrogen line, using only SS tubing, due to the corrosive properties of the H₂SO₄ packing.

The resulting gas is called femtograde nitrogen throughout this methodology.

5. Procedure

5.1. Extraction - Fish

- 5.1.1. Whole fish are received frozen from the field. After thawing the fillet or organ to be analyzed is homogenized and transferred to a dry, solvent-rinsed glass jar with foil-lined screw cap.
- 5.1.2. Weigh 20 g of fish homogenate into 250 ml Erlenmeyer flask.
- 5.1.3. Add 70 ml of 12N HCl and digest by shaking on wrist action shaker for 16 hours.
- 5.1.4. Add 1 ml of TCDD internal solution (4.1.3.) to each sample
- 5.1.5. Add 50 ml of hexane and extract for 2 hours on wrist action shaker.
- 5.1.6. Transfer hexane to 250 ml round-bottom flask, using a disposable pipette.
- 5.1.7. Repeat extraction twice more with 50 ml portions of hexane, shaking for 1 hour each time.
- 5.1.8. Evaporate combined extracts down to 20 ml, using a rotary evaporator and vacuum.
- 5.1.9. The extract is now ready for clean-up prior to GC/MS analysis.

NOTE: To ensure staff safety, extreme care must be taken in all transfer and evaporation steps, especially when handling concentrated solutions, which might contain TCDD.

5.2. Chromatographic packing - Preparation

- 5.2.1. Silicic acid

- 5.2.1.1. Silicic acid is dried in a glass tube (3.2.1.2.) at 180°C for 30 minutes under a continuous femtograde nitrogen purge.
- 5.2.1.2. Continue nitrogen purge during 15 minute cool down period.
- 5.2.1.3. Sequentially elute silicic acid with 150 ml portions of methanol and dichloromethane.
- 5.2.1.4. After all dichloromethane has eluted, return tube containing silicic acid to furnace set at 50°C and purge with femtograde nitrogen.
- 5.2.1.5. Over a period of 30 minutes, increase temperature to 180°C, venting gaseous effluent to a fumehood.
- 5.2.1.6. Further activate the packing at 180°C for 90 minutes, always purging with nitrogen.
- 5.2.1.7. Continue nitrogen purge during cool-down period.
- 5.2.1.8. Transfer activated silicic acid to a dry, solvent-rinsed bottle for storage in a desiccator, over silica-gel.

5.2.2. **Silver nitrate packing**

A 10% AgNO_3 on silicic acid packing is prepared as follows:

- 5.2.2.1. Weigh 6.0 g of AgNO_3 .
- 5.2.2.2. Dissolve AgNO_3 in 23 ml of deionized water.
- 5.2.2.3. Weigh 53.0 g of activated silicic acid (5.2.1.) into a 125 ml Erlenmeyer flask.
- 5.2.2.4. Add dissolved AgNO_3 to flask containing silicic acid and shake by hand until powder is free-flowing.
- 5.2.2.5. Allow packing to stand for 30 minutes at room temperature.
- 5.2.2.6. Transfer packing to glass tube (3.2.1.2.) plugged with glass wool and place in tube furnace at 70°C under a continuous flow of femtograde nitrogen.
- 5.2.2.7. Over a 5-hour period, increase temperature, stepwise, to 120°C.
- 5.2.2.8. After condensation of deionized water has ceased, activate packing at 125°C for 15 hours. Continue nitrogen purge during cool-down period.
- 5.2.2.9. Transfer cool packing to an amber glass bottle and store in a desiccator, over silica-gel.

5.2.3. Sulphuric acid/silicic acid packing

5.2.3.1. Weigh 42.0 g of activated silicic acid (5.2.1.) into a 250 ml Erlenmeyer flask.

5.2.3.2. Measure 33 ml of concentrated H_2SO_4 .

5.2.3.3. Carefully add the H_2SO_4 to the silicic acid in the flask and stopper.

5.2.3.4. Shake vigorously by hand until powder is free-flowing.

The above packing (42 g of silicic acid/33 ml of H_2SO_4) contains 44% H_2SO_4 .

For 22% H_2SO_4 /silicic acid use 58.5 g of silicic acid and 16.5 ml of H_2SO_4 .

NOTE: After treatment with H_2SO_4 , the silicic acid retains the corrosive properties of the acid and should be handled with great care.

5.2.4. Alumina packing

5.2.4.1. Pour alumina (4.2.5.) into the glass-wool plugged tube (3.2.1.2.) and dry in tube furnace at 300°C for 60 minutes under a continuous purge of femtograde nitrogen.

5.2.4.2. Cool to room temperature continuing nitrogen purge.

5.2.4.3. Elute 150 ml of dichloromethane through the packing.

5.2.4.4. Return tube to furnace, re-establish femtograde nitrogen purge.

5.2.4.5. Slowly increase temperature to 180°C, over a period of 30 minutes and maintain until all dichloromethane has vented.

5.2.4.6. Increase temperature slowly to 300°C and maintain for 90 minutes.

5.2.4.7. Cool packing under continuous nitrogen purge.

5.2.4.8. Transfer packing to glass bottle and store in desiccator over silica-gel.

5.3. Clean-up - Fish

An exhaustive clean-up procedure is followed which involves two-phase, dual column chromatography followed by high-performance-liquid chromatography (HPLC).

5.3.1. A column (3.3.1.) fitted with a reservoir, and plugged with glass wool is packed successively with 44% H_2SO_4 /silicic acid (5.2.3.) to a height of 16 cm, and sufficient 22% H_2SO_4 /silicic acid (5.2.3.) to

extend into the reservoir, to a height of 2.5 - 5.0 cm, depending on fat content of sample.

- 5.3.2. The second column (3.3.2.) is packed to a height of 18 cm with basic alumina (4.3.3.) and is connected to the bottom of the H_2SO_4 column.
- 5.3.3. Apply sample extract (5.1.8.) to top of H_2SO_4 column.
- 5.3.4. Rinse sample container with 10 ml of hexane, add rinsing to the column.
- 5.3.5. Rinse sample container with 30 ml of hexane, add rinsing to the column and allow to drain dry.
- 5.3.6. Remove H_2SO_4 column and connect clean reservoir to alumina column.
- 5.3.7. Elute with 100 ml of hexane, allowing eluant to reach top of packing.
- 5.3.8. Immediately elute with 30 ml of dichloromethane, collecting eluate in a 50 ml centrifuge tube.
- 5.3.9. Blow-down eluate, carefully, to dryness, using femtograde nitrogen.
- 5.3.10. Make up residue in 1 ml of hexane for second clean-up phase.
- 5.3.11. Plug column (3.3.8.) with glass wool and fill with AgNO_3 /silicic acid (4.3.7.) to a height of 5 cm.
- 5.3.12. Into bottom of this column (5.3.11.) fit a column (3.3.2.) which has been plugged with glass wool and filled with basic alumina (4.3.3.) to a height of 21 cm.
- 5.3.13. Apply sample (5.3.10.) to top column using a disposable pipette.
- 5.3.14. Rinse sample container with 4 x 1 ml of hexane, adding each rinse to the column.
- 5.3.15. Elute with 100 ml of hexane until top column is dry.
- 5.3.16. Remove top column, insert reservoir into second (alumina) column.
- 5.3.17. Sequentially wash column with 20 ml of 10% CCl_4 /hexane and 10 ml of hexane.
- 5.3.18. Elute with 25 ml of CH_2Cl_2 and collect in a 50 ml centrifuge tube.
- 5.3.19. Blow-down to dryness, using femtograde nitrogen.
- 5.3.20. Make up residue in 50 μl of methanol for HPLC clean-up.
- 5.3.21. Take up 50 μl of sample (5.3.20.) into a 100 μl syringe.
- 5.3.22. Rinse sample container twice, with 15 μl portions of methanol and take up into syringe containing the sample.

- 5.3.23. Inject whole 80 μ l (combined rinses and sample) into HPLC.
- 5.3.24. Collect TCDD fraction, as determined by previous calibration with 2,3,7,8-TCDD, (usually about 2 ml in the 13 - 15 minute retention range) in a 10 ml volumetric flask containing 1 ml of hexane.
- 5.3.25. Partition TCDD into hexane by shaking with 6 ml of NaHCO_3 (4.3.12).
- 5.3.26. Transfer top hexane layer to a 0.1 ml Reacti-vial as follows:
 - add hexane dropwise to the Reacti-vial
 - evaporate gently under a stream of femtgrade nitrogen
 - repeat dropwise additions and evaporations until all hexane has been transferred.
- 5.3.27. Repeat partition with another 1 ml of hexane.
- 5.3.28. Transfer hexane to Reacti-vial, as described (5.3.26.).
- 5.3.29. Evaporate to dryness and make-up in 10 μ l of isooctane for GC analysis.

5.4. Screening/confirmation

The final identification/quantitation step involves detection of 2,3,7,8-TCDD by monitoring ions at m/z 319.9, 321.9 and 323.9 at the correct retention time. The retention time is established daily by calibration using a 20pg TCDD solution. Sensitivity is 10 pg under normal operating conditions (3.4.1. and 3.4.2.). In fish, GC/MS quantitation includes monitoring m/z 335.9 for C^{13} labelled 2,3,7,8-TCDD. The recovery of C^{13} 2,3,7,8-TCDD is calculated for each sample and the TCDD level is adjusted accordingly.

5.4.1. GC/screening

- chromatographic peak must elute within ± 0.02 minute of the standard retention time.
- integrated peak areas from each capillary column must agree within $\pm 20\%$.

Samples meeting both these criteria are further analyzed by mass spectrometry.

5.4.2. GC/MS Confirmation

Samples must meet the following criteria for confirmation as 2,3,7,8-TCDD:

1. Correct capillary column (ECD and/or GC/MS) retention time of 2,3,7,8-TCDD.
2. Correct chlorine isotope ratios of m/z 319.9/321.9/323.9 within 15% of theoretical ratios of 76:100:48.
3. Good (greater than 65%) recovery for C^{13} TCDD internal standard; proper retention time for C^{13} TCDD at m/e 335.9.

4. Response of m/e 319.9 and 321.9 must be greater than 2.5 times the noise level.

6. **Calculations and Reporting**

Results are reported as 2,3,7,8-TCDD.

Results are obtained by comparison with a standard solution (4.2.2.2.).

7. **Precision and Accuracy**

The recovery of 2,3,7,8-TCDD from a fish fortified with 100 pg/g was 83%.

8. **Bibliography**

L.L. Lamparski, T.J. Neshick, R.H. Stehl. Anal. Chem. 51 1453 (1979).
Determination of Part-per-Trillion Concentrations of 2,3,7,8-Tetrachlorodioxin in Fish.

THE DETERMINATION OF CHLOROPHYLLS

Chlorophyll enables photosynthesis to occur, and consequently is found in significant amounts in plant tissue. Chlorophyll a and b measurements in river and lake samples can be used for estimating the standing stock of algae in natural bodies of water, and for assessing the trophic status of lakes and streams. Excessive nutrient release into water bodies may stimulate large increases in biomass leading to accelerated eutrophication of the water body. A nutrient removal scheme is often implemented to control biomass growth and chlorophyll determinations on water samples from these areas can monitor the scheme's success.

Sample Handling and Preservation

Glass bottles are recommended for sample collection.

A 0.5% magnesium carbonate suspension should be added to the sample (approximately 1 ml per liter of sample). This helps to stabilize the chlorophyll by keeping the sample basic. Chlorophylls are rapidly converted to pheopigments under acidic conditions.

Preferably, samples should be filtered through 1.2 μ m membrane filters at the time of collection. The moist filters should then be placed in labelled petri dishes on a piece of regular filter paper which absorbs excess water. The filter may be shipped without refrigeration but must be covered with aluminum foil to exclude light. Samples must not be stored for extended periods.

If filtration in the field is not possible, samples should be refrigerated and kept in the dark to reduce further photosynthesis. Samples should be analyzed as soon as possible.

Selection of Method

A spectrophotometric method is currently employed for chlorophyll determination. Chlorophyll is extracted from plant tissue using a 90% acetone solution and absorbances are determined at 750, 665, 645 and 630 nm. The UNESCO equations are used to calculate chlorophyll a and b. Any other pigments or degradation products which absorb at these wavelengths will be included as interferences. Degradation products may be separated from the chlorophylls by specialized techniques (8.7, 8.13, 8.20).

The bibliography contains many general references on the test, some of which include modifications for special applications.

CHLOROPHYLLS

Spectrophotometry Method A

SUMMARY

Matrix	This method is routinely used on plant material filtered from river and lake samples.
Substance determined.	Chlorophylls a and b, and acidified chlorophyll a are reported.
Interpretation of results.	Results are reported in $\mu\text{g/l}$. Chlorophyll results are used to estimate the biomass of standing algae crops in natural waters.
Principle of method.	An aliquot of sample is filtered through a $1.2\ \mu\text{m}$ cellulose nitrate membrane filter and the residue is extracted with 90% acetone. Absorbances are recorded at 750, 665, 645 and 630 nm using a spectrophotometer, and the results for chlorophylls a and b are calculated using the UNESCO equations.
Time required for analysis.	A single analysis requires approximately 24 hours. Eighty samples may be tested concurrently.
Range of application.	From $2\ \mu\text{g/l}$ to $20\ \mu\text{g/l}$ chlorophyll on a 1 liter sample.
Standard deviation.	Not yet available.
Accuracy.	Accuracy data using absolute standards which would involve both the extraction and measurement steps are not yet available.
Detection criteria.	Not yet available.
Interferences and shortcomings.	The degradation of chlorophyll a to pheophytin a is inhibited by using approximately 1 ml of 0.5% magnesium carbonate as a preservative. Turbidity effects are removed by subtracting the absorbances at 750 nm from all other absorbances (8.19). Excessive filter pressure ruptures phytoplankton cells with a subsequent loss of chlorophyll through the filter. Exposure of the extracted chlorophyll to bright light will cause the color to fade.
Minimum volume of sample.	Depends on nature of sample and concentration of algae. A 1 liter sample is required at the $2.0\ \mu\text{g/liter}$ chlorophyll level.

**Preservation and
sample container.**

Glass bottle sample containers are recommended. Preserve with approximately 1 ml of 0.5% magnesium carbonate per liter of sample (avoid excess), refrigerate, store in the dark, and analyze immediately. Frozen samples may be stored up to 30 days when kept in the dark. If samples are to be filtered at the time of sampling, preserve with 1 ml of 0.5% magnesium carbonate prior to filtration and submit samples as filter cakes in labelled petri dishes covered in aluminum foil with a record of the original sample volumes. Store at -20°C and analyze as soon as possible.

**Safety
considerations.**

Use the acetone reagent in a well ventilated room. Prolonged inhalation of acetone vapors is harmful. Acetone vapor is highly flammable. Do not use near an open flame.

CHLOROPHYLLS

Spectrophotometry Method A

1. Introduction

Suspended plant material is removed by filtration through a cellulose nitrate membrane filter. The filter is dissolved in 90% acetone, and glass beads are placed in the solution to pulverize the plant cells during vigorous vibration on a Vortex Jr. mixer. The acetone solution containing chlorophyll is then filtered to remove debris. Absorbances for the extracts are recorded at 630, 645, 665 and 750 nm using a spectrophotometer. The UNESCO equations are used to calculate the concentrations of chlorophylls a and b, respectively.

2. Interferences and Shortcomings

- 2.1. **Low pH** of samples causes each molecule of chlorophyll a to lose a magnesium atom, and undergo degradation to pheophytin a with an accompanying shift in absorption maxima. This interference is overcome by the addition of 1 ml of 0.5% magnesium carbonate per liter of sample. This will insure that the pH remains high enough to retard degradation. Too much preservative, however, interferes by absorbing small amounts of pigment, clogging the filter and increasing the turbidity.
- 2.2. **Turbidity** interferences are minimized by extract filtration to remove suspended particulate matter. Any remaining turbidity can be reduced by centrifuging the sample if it causes an abnormal scan trace. A blank correction is made by subtracting the absorbance reading at 750 nm from each of the three previously mentioned absorbances.
- 2.3. **Excessive Filter Pressure** (greater than 30 mm Hg) causes rupturing of the phytoplankton cells with a subsequent loss of sample through the filter. Careful regulation of the filter pressure will prevent this loss.
- 2.4. **Bright Light** can cause fading of the chlorophyll in the acetone extract. Storage of the acetone extract in a cool dark place will minimize this effect.

3. Apparatus

- 3.1. Millipore filtering funnel assembly.
- 3.2. Clinical centrifuge.
- 3.3. Membrane filters, Sartorius cat. no. SM-11303, 47 mm pore size 1.2 μ m.
- 3.4. Centrifuge tubes with caps, graduated, 12 ml (56).
- 3.5. Funnels, long stemmed (28).

- 3.6. Filter paper, glass fiber, 9.0 cm.
- 3.7. Petri dishes and filter pads (28).
- 3.8. Spectrophotometer, (Unicam SP 1800) with a chart recorder.
- 3.9. Aluminum foil.
- 3.10. Mixer, Vortex Junior or tissue grinder.
- 3.11. Graduated cylinders, 250 ml, 1000 ml.
- 3.12. Glass beads, 0.25 inch diameter.
- 3.13. Dispensette bottle.

4. Reagents

- 4.1. Acetone, reagent grade.
- 4.2. Hydrochloric acid, reagent grade.
- 4.3. **Acetone Solution (90%)**

Dilute 900 ml of acetone to 1 liter with distilled water and mix thoroughly.

NOTE: Prepare in a well ventilated room away from open flames. Store the acetone reagent in a steel safety container which will prevent explosions resulting from vapour ignition.

- 4.4. **Hydrochloric Acid Solution (10%)**

In a volumetric flask dilute 100 ml of concentrated hydrochloric acid with 900 ml of distilled water and mix thoroughly.

NOTE: Wear eye protection and gloves when preparing this acid solution.

5. Procedure

IF SAMPLE HAS BEEN PREFILTERED BEGIN AT STEP 5.2.

5.1. Sample Filtration

- 5.1.1. Select an appropriate aliquot of the sample, considering the amount of chlorophyll and particulates. Pour a sample aliquot into a graduated cylinder. Record to the nearest 10 ml the amount of sample filtered.
- 5.1.2. Filter the sample through a 47 mm cellulose nitrate membrane filter (pore size 1.2 μm) using a vacuum filtration apparatus.
NOTE: Do not use excessive vacuum pressure.
- 5.1.3. Place the filter on a filter pad in a labelled petri dish. Cover with aluminum foil or store in the dark.

5.2. Sample Extraction and Measurement

- 5.2.1. Fold the slightly moist membrane filter four times and insert into a 12 ml centrifuge tube. Add approximately 3 glass beads.
- 5.2.2. Add 9 ml of 90% acetone using a dispensette bottle.
- 5.2.3. Disintegrate algae cells to liberate the chlorophyll pigment by vigorous shaking using a Vortex Junior mixer or a tissue grinder until the filter disintegrates.
- 5.2.4. Cap centrifuge tube, cover with aluminum foil and allow to soak 18 - 24 hours in the dark at 4°C.
- 5.2.5. Filter extract through a 9 cm glass fiber filter on a long stemmed funnel into a graduated centrifuge tube. Wash filter with 90% acetone until all green pigment is removed.
NOTE: If the residue on the filter remains green, analyze the portion extracted and report answer as greater than the value obtained.
- 5.2.6. Adjust final volume of the extract to 12.0 ml with 90% acetone, cap and mix thoroughly by inverting several times.
- 5.2.7. Pour the extract into a 4 cm cuvette. Place the cuvette in the spectrophotometer and record the absorbances at 750, 665, 645, and 630 nm on a preset scan range from 590 to 760 nm using a 90% acetone blank. Centrifuge the extract if the absorbance at 750 nm is greater than 20 lines on the chart.
NOTE: Absorbance at 750 nm is very sensitive to changes in the acetone water proportions. Use exactly 90% acetone.
- 5.2.8. To obtain an acidified chlorophyll a result, add one drop of 10% hydrochloric acid to the sample in the cuvette, mix thoroughly and let stand for 1 minute then repeat the scan.

6. Calculation and Reporting

The concentrations of chlorophylls a and b, and acidified chlorophyll a are calculated using the UNESCO equations (8.19).

$$\text{chlorophyll a } \mu\text{g/l} = \left(11.64A_{665}(\text{cor}) - 2.16A_{645}(\text{cor}) + 0.1A_{630}(\text{cor}) \right) \frac{X}{L} \times \frac{1}{Y}$$

$$\text{chlorophyll b } \mu\text{g/l} = \left(-3.94A_{665}(\text{cor}) + 20.97A_{645}(\text{cor}) - 3.66A_{630}(\text{cor}) \right) \frac{X}{L} \times \frac{1}{Y}$$

$$\text{acidified chlorophyll a } \mu\text{g/l} = \left(26.73 (A_{665}(\text{cor}) - A_{665a}(\text{cor})) \right) \frac{X}{L} \times \frac{1}{Y}$$

Where:

A_n = absorbance at wavelength n .

$A_n(\text{cor})$ = absorbance at wavelength n corrected for turbidity

$A_{na}(\text{cor})$ = acidified absorbance at wavelength n corrected for turbidity

L = original sample volume in liters

X = extract volume in ml

Y = cuvette length in cm (path length)

n = wavelength in nm.

Turbidity Correction:

$$A_n - A^{750} = A_n(\text{cor})$$

Final chlorophyll results are reported to the nearest 0.1 $\mu\text{g/l}$ in the 0.1 - 9.9 $\mu\text{g/l}$ chlorophyll range and to the nearest 1 $\mu\text{g/l}$ in the 10 - 99 $\mu\text{g/l}$ chlorophyll range. All results are reported to 2 significant figures.

7. Precision and Accuracy

Absolute standards involving both the extraction and measurement steps are not yet in use in this laboratory.

8. Bibliography

- 8.1 American Public Health Association, American Water Works Association, Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater. 13th ed., APHA, Washington, D.C. 746-747.
- 8.2 Crietz, G.E. and Richards, F.A. (1955). The estimation and characterization of plankton populations by pigment analysis. *Journal of Marine Research*, 11(2): 147-155.
- 8.3 Duxbury, A.C. and Yentsch, C.S. (1956). Plankton pigment nomographs. *Journal of Marine Research*, 15(1): 92-101.
- 8.4 Eaton, J.W. and Moss, B. (1966). The estimation of numbers and pigment content of epipelagic algal populations. *Limnology and Oceanography*, 11: 584-494.
- 8.5 Laesso, A., Hasen, Vagn, Kr. (1961). Ultrasonic Extraction of Chlorophyll "a" from Phytoplankton. Plankton Committee Report No. 143. Cons. Internat. Explor. de la Mer.
- 8.6 Livingston, R.R., Pariser, R., Thompson, L. and Weller, A. (1953). Absorption spectra of solutions of pheophytin a in methanol containing acid or base. *Journal of the American Chemistry Society*, 75: 3025-3026.
- 8.7 Lorenzen, C.J. (1967). Determination of chlorophyll and pheopigments, spectrophotometric equations. *Limnology and Oceanography*, 12: 343-346.
- 8.8 Marker, A.F.H. (1972). The use of acetone and methanol in the estimation of chlorophyll in the presence of pheophytin. *Freshwater Biol.*, 2: 361-385.

- 8.9. Moss, B. (1967). A spectrophotometric method for the estimation of percentage degradation of chlorophylls to pheo-pigments in extracts of algae. *Limnology and Oceanography*, **12**: 335-340.
- 8.10. Moss, B. (1967). A note on the estimation of chlorophyll "a" in freshwater algal communities. *Limnology and Oceanography*, **12**: 341-343.
- 8.11. Odum, H.T., McConnell, W. and Abbott, W. (1958). The chlorophyll "a" of communities. Publication of the Institute of Marine Science, Texas, **5**: 65-96.
- 8.12. Parsons, T.R. (1961). On the pigment composition of eleven species of marine phytoplankters. *Journal of the Fisheries Research Board of Canada*, **18**: 1017-1025.
- 8.13. Parsons, T.R. and Strickland, J.D.H. (1963). Discussion of the spectrophotometric determination of marine plant pigments with revised equations for ascertaining chlorophylls and carotenoids. *Journal of Marine Research*, **11**: 156-172.
- 8.14. Richards, F.A. (1952). The estimation and characterization of plankton populations by pigment analysis I. The absorption spectra of some pigments occurring in diatoms, dinoflagellates and brown algae. *Journal of Marine Research*, **11**(2): 147-155.
- 8.15. Richards, F.A. and Thompson, T.G. (1952). The estimation and characterization of plankton populations by pigment analysis II. A spectrophotometric method for the estimation of plankton pigments. *Journal of Marine Research*, **11**(2): 156-172.
- 8.16. Strickland, J.D.H. and Parson, T.R. (1965). A Manual of Sea Water Analysis. *Bulletin of Fisheries Research Board of Canada*, **125**, 2nd ed., 203.
- 8.17. Talling, J.F. and Driver, D. (1961). Some problems in the estimation of chlorophyll "a" in phytoplankton. In: *Proceedings of the Conference of Primary Production Measurement of Marine Freshwater*. M.S. Doty (ed.). University of Hawaii, U.S. Atomic Energy Commission Publication TID 7633, 142-146.
- 8.18. Tucker, A. (1949). Pigment extraction as a method of quantitative analysis of phytoplankton. *Transactions of the American Microscopy Society*, **68**: 21-33.
- 8.19. United Nations Educational, Scientific and Cultural Organization (1966). *Monographs on Oceanographic Methodology*, 1. Determination of Photosynthetic Pigments in Sea Water. UNESCO, Paris, 69 p.
- 8.20. Vernon, L.P. (1960). Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. *Analytical Chemistry*, **32**: 1144-1150.

THE DETERMINATION OF HEXAVALENT CHROMIUM

Chromium and its salts are used extensively in the manufacture of steel alloys, as paint pigments, dye mordants and in electroplating solutions. Chromates are frequently added to cooling water for corrosion control.

Hexavalent chromium has carcinogenic properties, hence the necessity for monitoring its concentration in water. The maximum permissible concentration (8.5, 8.6) of the hexavalent form in drinking water is 0.05 mg/l.

Sample Handling and Preservation

Chromate ions have a tendency to be adsorbed on the surface of the container, and may also be reduced by various agents. For this reason new bottles rather than old etched containers should be used for sample collection.

No preservative should be used. Glass bottles with plastic lined caps are acceptable. In all cases the bottle should be filled to the top.

Samples for hexavalent chromium should be tested on the day of collection. Storage for more than two or three days is not recommended.

Selection of Method

Method A, a diphenylcarbazide spectrophotometric procedure, is applicable to the determination of hexavalent chromium present in a natural or treated water.

A method for total chromium is included under Determination of Trace Metals by Atomic Spectroscopy.

HEXAVALENT CHROMIUM

Diphenylcarbazide Spectrophotometric Method A

SUMMARY

Matrix.	This method is used routinely on natural or treated waters.
Substance determined.	Hexavalent chromium, Cr VI.
Interpretation of results.	Hexavalent chromium is measured, and results reported as mg/lCr.
Principle of method.	Hexavalent chromium reacts with diphenylcarbazide in slightly acid solution, to produce a reddish purple colour. The spectrophotometric absorbance of the aqueous solution is measured at 540 nm.
Time required for analysis.	A single hexavalent chromium test can be determined in 15 minutes. Approximately 60 analyses can be performed in a day.
Range of application.	The absorption curve obeys Beer's Law over the range (0.02 to 0.50 mg/l). Dilution or concentration may be used to extend the range.
Standard deviation.	0.04 mg/l for 30 determinations on a sample containing 0.80 mg/l chromium.
Accuracy.	Not available.
Detection criteria.	.01 mg/l.
Interferences and shortcomings.	Mercury produces a blue color, molybdenum (VI) a violet colour and vanadium (V) a yellow brown color. The reaction for mercury is not very sensitive at the acidity employed. In order to minimize the interference from vanadium, a time lapse of 10 minutes after addition of the diphenylcarbazide reagent is allowed, to permit fading of the vanadium complex. At levels up to 5 mg/l iron interference may be suppressed by the addition of phosphoric acid. At higher levels, iron can be precipitated with ammonium hydroxide after converting chromium to the hexavalent form. Highchloride content can result in loss of chromium as chromyl chloride in the digestion process.

**Minimum volume
of sample.**

100 ml.

**Preservation and
sample container.**

Samples for hexavalent chromium must not be preserved in any way and should be analyzed within a day of collection. Plastic bottles are preferred but glass bottles with plastic-lined caps are acceptable. In all cases the bottle should be filled to the top.

**Safety
considerations.**

Use normal safety precautions when handling acids.

HEXAVALENT CHROMIUM

Diphenylcarbazide Spectrophotometric Method A

1. Introduction

Diphenylcarbazide reacts with hexavalent chromium in slightly acidic solution to give a reddish purple color and the absorption of this colored solution is determined spectrophotometrically at 540 nm.

In the determination of hexavalent chromium the sample solution must be clear. About 100 ml of sample should be filtered or coagulated in order to obtain a clear aliquot.

2. Interferences and Shortcomings

The diphenylcarbazide reaction is almost specific for hexavalent chromium Cr VI, but certain elements can interfere, for example, mercury produces a blue color, molybdenum VI produces a violet color and vanadium V produces a yellow brown color. These elements are not commonly found in ordinary water and wastewater samples. However, some protection against their effects is built into the procedure. After addition of the diphenylcarbazide reagent a time lapse of 10 minutes is allowed before reading to permit the fading of these interfering colors to an insignificant level.

Iron is a more serious and commonly occurring interference. At levels up to 5 mg/l iron, addition of phosphoric acid is sufficient to suppress the interference. At higher levels iron is precipitated by the addition of ammonium hydroxide to make the sample alkaline. The sample should be reacidified before addition of diphenylcarbazide.

Organic material is liable to interfere with the reaction in two ways. A positive interference in which the organic material also exhibits spectrophotometric absorbance is prevented by preparing a blank containing the original sample without the reagent and carrying it through the procedure. A negative interference is a more serious problem involving the possible reduction of hexavalent chromium by the organic material. Therefore, it must be stipulated that paper cannot be used as a filtration medium in the sample preparation.

3. Apparatus

- 3.1. Spectrophotometer, B & L Spectronic 100 (capable of being set at 540 nm).
- 3.2. Nessler Tubes, Pyrex glass, 50 ml (24).
- 3.3. Erlenmeyer flasks, Pyrex glass 125 ml (24).

3.4. Various pipettes.

NOTE: All glassware must be acid rinsed prior to use. It should be noted that chromic acid must not be used in the cleaning process.

4. Reagents

4.1. Potassium dichromate ($K_2Cr_2O_7$), reagent grade crystals.

4.2. Sulphuric acid (H_2SO_4), concentrated, "Aristar", (high purity grade low in metals).

4.3. Diphenylcarbazide ($(C_6H_5.NH.NH)_2 CO$), reagent grade.

4.4. Acetone, reagent grade.

4.5. Ammonium hydroxide (NH_4OH), concentrated, reagent grade.

4.6. Dilute Sulphuric Acid (1:1 v/v)

Carefully add concentrated sulphuric acid to an equal volume of distilled water, while cooling. Exercise caution since much heat is generated by this dilution.

NOTE: Wear eye and hand protection.

4.7. Diphenylcarbazide Solution (0.5%) in Acetone

Dissolve 1.25 g of diphenylcarbazide in 250 ml acetone. Store in a low actinic glass bottle. As long as the solution remains colorless it may be used but it should be discarded when appreciable color develops. (8.1).

4.8. Stock Chromium Solution (100 mg/l Cr)

Dissolve 0.2828 g anhydrous potassium dichromate in distilled water and dilute to 1 liter in a volumetric flask. Store in a Pyrex reagent bottle.

4.9. Standard Chromium Solution (10 mg/l Cr)

Dilute 10.0 ml stock chromium solution to 100 ml in a Pyrex volumetric flask.

4.10. Working Standards

Working standards are prepared by diluting suitable aliquots of standard chromium solution with distilled water to give a set of standards with chromium concentrations between 0.01 and 1.0 mg/l.

5. Procedure

5.1. Filter or coagulate approximately 100 ml of sample in order to obtain a clear effluent.

NOTE: Glass fibre filter should be used to avoid risk of reducing Cr VI. Any coagulation aids used should not reduce Cr VI.

5.2. Measure an aliquot of sample, 50 ml or less, depending on the concentration of chromium present and the amount of colored or turbid material present in the sample. If sample is colored or turbid use a portion of the sample in the blank to compensate for interference in reading the absorbance of the unknown.

- 5.3. Add 2 ml of 1:1 v/v sulphuric acid to the sample and blank.
- 5.4. Add 1.0 ml diphenylcarbazide solution, mix, and allow to stand for 10 minutes before reading at 540 nm in a Spectrophotometer.
- 5.5. A standard curve is prepared by performing the above procedure on standard chromium solution (from 1 to 40 ug Cr VI in tube).

6. Calculation and Reporting

The chromium content of the sample aliquot is read from the standard curve, and the chromium concentration in the sample is calculated as follows:

$$\text{mg/l Cr VI} = \frac{\mu\text{g Cr}}{\text{ml sample}}$$

Range in mg/l:	Report in mg/l to:
0.02	0.02
0.02 to 0.09	1 significant figure
0.10 to 9.9	2 significant figures
10 to 100	2 significant figures
100 to 1000	2 significant figures

7. Precision and Accuracy

According to results from 30 determinations obtained using the above method to analyze a sample containing 0.8 mg/l in chromium, the standard deviation was 0.04 mg/l. Two long term standards at approximately 20% and 80% of scale are used for controls. Absorbance must be within 10% of the established values for the experiment to proceed..

8. Bibliography

- 8.1 Allen, T. L. (1958). Microdetermination of chromium with 1. S-diphenylcarbohydrazide. *Analytical Chemistry*, **30** (3): 447-450.
- 8.2 American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). *Standard Methods for the Examination of Water and Wastewater*, 13th ed. APHA, Washington, D.C.
- 8.3 Analytical Reference Service (1968). *Water Metals No. 4 Study No. 30*. U.S. Department of Health, Education and Welfare, Cincinnati, Ohio, 117 p.
- 8.4 American Society for Testing Materials (1969). *Total chromium in water. Water, Atmospheric Analysis, Part 23, ASTM Standards*, 467-473.
- 8.5 Federal Water Pollution Control Association (1968). *Report of the Committee on Water Quality Criteria*. FWPCA, U.S. Department of the Interior, Washington, D.C.
- 8.6 Ontario Water Resources Commission. (1967). *Guidelines and Criteria for Water Quality Management in Ontario*. OWRC.

THE DETERMINATION OF APPARENT COLOR

Color of natural surface water is associated with the presence of humic and fulvic acids, tannins and lignins derived from aquatic and terrestrial plant material. Lakes which contain significant amounts of these colored substances are commonly referred to as "acid bog" or "brown-water" lakes. In ground waters, the color is more likely due to the presence of natural metallic ions, predominantly iron and manganese. In either case, the color is characteristically manifested as varying hues of yellowish brown.

Naturally colored waters are harmless but undesirable for drinking and recreation. Color guidelines have been set for drinking waters and are based primarily on aesthetic considerations. The Ontario Ministry of the Environment recommends that the color of drinking waters should not exceed 5 color units and water taken for treatment should not exceed 75 color units (prior to treatment). Color caused by the presence of iron and manganese may cause taste and staining problems. Waters having colors of unusual hues, such as those of industrial origin, may differ greatly from the typical yellowish-brown hues of natural water and cannot properly be assessed by this method. The color dilution test is normally used when evaluating industrial wastes.

Apparent color as measured by this method, measures the color due to dissolved substances and suspended matter. True color, however considers the color after turbidity removal. Apparent color is more representative of the color actually perceived when the water is viewed.

Sample Handling and Preservation

The apparent color of water is greatly dependent on pH, the extent of biological activity and the level of particulate present. In order to minimize changes during transit, sample containers should be filled to the neck, sealed tightly and refrigerated until the test is performed. Extended delays between sampling and analysis lead to non-representative results.

Selection of Method

Two methods are currently used for apparent color determinations. Method A is a spectrophotometric method involving the calibration of a spectrophotometer using Hazen's cobalti-platinate standards ($1 \text{ mg/l Pt} = 1 \text{ Hazen unit}$) which are dilutions of a stock potassium platinum chloride solution, concentrated hydrochloric acid and cobalt chloride (8.3). The absorbances of a sample are measured colorimetrically using a broad band filter with transmission limits of 400 - 450 nm and one with limits of 660 - 740 nm. The latter is required to obtain a suitable correction for turbidity and particulates. This method retains the familiar Hazen Unit expression of color yet results obtained are independent of operator.

Method B, a visual comparison test is used primarily in the field or when the necessary apparatus for Method A is unavailable. The unfiltered sample color is visually compared with a set of pre-calibrated colored discs and the color expressed as Hazen units. Alternatively, the analyst may prepare chloroplatinate standard solutions for comparison with the samples.

APPARENT COLOR
Spectrophotometric Method A

SUMMARY

Matrix.	This method is used routinely on drinking water, river and lake samples.
Substances determined.	Apparent color in Hazen units.
Interpretation of results.	Apparent color is a physical measurement which considers the combined effect of most colored components in natural waters. The most prevalent substances include humic acids, tannins, lignins, fulvates, iron and manganese. This test is not generally applicable to industrial waste samples.
Principle of method.	The absorbance of an unfiltered sample is measured colorimetrically using 2 broad band filters, one at 400 - 450 nm and one at 660 - 740 nm
Time required for analysis.	Approximately 2 minutes are required for a single analysis. On a routine basis, about 100 tests can be performed in a day.
Range of application.	Color is reported in the 1 - 100 Hazen unit range without dilution. If instrument readings exceed 70 with the 400 - 450 nm filter or 20 with the 660 - 740 nm filter, the sample is centrifuged; and the color of the supernatant is measured and reported as "true color".
Standard deviation.	1 H.U. in the 1 - 100 H.U. range.
Accuracy.	The correlation between this method and the visual comparison Method B exceeds 95% for routine domestic water samples.
Detection criteria.	1.0 H.U.
Interferences and shortcomings.	Changes in pH, biochemical activity and iron and manganese precipitation affect sample color. This method is not applicable to colors other than those derived from natural sources. Equation factors for the calculation of color vary with instrument and age of the phototube.
Minimum volume of sample.	75 ml.

**Preservation and
sample container.**

To minimize color change, sample bottles should be filled to capacity, sealed tightly and refrigerated until the test is performed. Immediate delivery to the laboratory is essential. Glass bottles are preferred.

**Safety
considerations.**

None.

APPARENT COLOR

Spectrophotometric Method A

1. Introduction

The apparent color of a well-mixed unfiltered sample is calculated from readings obtained on a Klett-Summerson colorimeter using 2 broad band filters, #42 (400 - 450 nm) and #69 (660 - 740 nm); these measurements are directly related to absorbance values. The use of a 400 - 450 nm broad band filter eliminates the problem of selecting a single wavelength for color measurement while maintaining comparability with the Hazen disc procedure (Method B). Since the presence of particulates results in high estimates of color by spectrophotometric methods, a turbidity correction is required. The optical density of Hazen color standards is negligible above 600 nm and therefore the sample absorbance is measured using filter #69 and this value (after multiplication by a slope correction factor) is subtracted from the measurement on the #42 filter.

2. Interferences and Shortcomings

Sample color may be affected by a variety of chemical and biological phenomena including changes in pH, biochemical oxidation or reduction and precipitation of iron and manganese compounds. Proper handling and preservation prior to analysis will minimize these problems. The equation factor given in section 6 may vary from instrument to instrument and with age of the phototube.

3. Apparatus

- 3.1. 2 Klett-Summerson photoelectric colorimeters; 4 cm path length.
- 3.2. Broad band filters, #42 (transmission limits of 400 - 450 nm) and #69 (transmission limits of 660 - 740 nm).
- 3.3. Solution cells.

4. Reagents

- 4.1. Potassium chloroplatinate (K_2PtCl_6), reagent grade.
- 4.2. Cobaltous chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), reagent grade crystals.
- 4.3. Hydrochloric acid (HCl), concentrated, reagent grade.
- 4.4. **Color Stock Solution (500 mg/l Pt)**

Dissolve 1.246 g potassium chloroplatinate and 1.00 g cobaltous chloride hexahydrate in 500 ml distilled water to which 100 ml concentrated hydrochloric acid has been added. Dilute to 1 liter with distilled water.

4.5. Working Standards

Depending on the expected color range of samples a series of working standards are prepared by diluting aliquots of stock solutions with distilled water. For the full 0 - 100 range, standards can be prepared by diluting 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ml aliquots of color stock solution to 50 ml with distilled water. This gives a series of standards with colors of 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 and 100 Hazen units. Usually not all these standards are required.

4.6. Quality Control Solutions

QC-A and QC-B solutions are prepared from a different 500 mg/l Pt color stock solution than that used for the working standards.

QC-A (25 H.U. - domestic waters):

Dilute 100 ml color stock solution to 2 liters with distilled water.

QC-B (2.5 H.U. - domestic waters):

Dilute 10 ml color stock solution to 2 liters with distilled water.

QC-A (50 H.U. - rivers and lakes):

Dilute 200 ml color stock solution to 2 liters with distilled water.

QC-B (25 H.U. - rivers and lakes):

Dilute 100 ml color stock solution to 2 liters with distilled water.

5. Procedure

- 5.1. For each colorimeter, ensure the appropriate filter is in place.
- 5.2. Adjust galvanometer needle to coincide with the line on the scale by turning the knob located just above the pointer.
- 5.3. Ensure that the short circuit switch on the right hand side of the instrument is on. When off, this switch protects the needle during transit.
- 5.4. Switch colorimeter lamps on and allow 10 minutes for instrument stabilization.
- 5.5. Employing the colorimeter with #42 broad band filter, calibrate with working standards in order to determine the factor required to convert instrument readings to Hazen units. Once this factor (≈ 1.35) has been determined for a given instrument, the daily quality control program ensures that the slope factor does not change unduly. However, this calibration procedure must be repeated whenever the colorimeter is overhauled or phototubes replaced.
- 5.6. Clean and fill solution cell with distilled water. Place cell in cell compartment such that path length is 4 cm, and set scale at zero. Adjust the ZERO ADJUSTMENT knob until needle coincides with the line on the blank pointer scale.
- 5.7. Fill solution cell with QC-A and place in compartment of first colorimeter (filter #42). (Throughout the color test, the path length is always 4 cm.) Turn SCALE knob until pointer returns to zero line. Confirm that scale reading conforms to limits based on previous experience. Repeat for second colori-

meter (filter #69); confirm that scale reading is negligible. Repeat both steps for QC-B. Record readings, and convert to Hazen units. If QC-A and QC-B do not meet specifications and problem cannot be solved, consult senior technician who will determine if system must be recalibrated.

- 5.8. Fill solution cell with sample and place in cell compartment of first colorimeter (filter #42). Turn SCALE knob until pointer returns to zero line. Record scale reading. Repeat for second colorimeter (filter #69). Record scale reading.
- 5.9. Analyze a blank (distilled water) after each group of 10 samples. Rezero the instrument if necessary.
- 5.10. If the scale readings for the system exceed 70 with filter #42 or 20 with filter #69, do not report results. Centrifuge the sample, and analyze the supernatant. If the above limits can be met with the supernatant, calculate the color result as usual but report the result as True Color in Hazen units. Dilutions with distilled water may be necessary to get the reading on the #42 filter less than 70.

6. Calculation and Reporting

Apparent color is calculated by an equation of the following form for the range 0 - 100 HU:

$$\text{Apparent color (Hazen units)} = C (A_{42} - 1.62 A_{69})$$

Where:

A_{42} = instrument reading using filter #42

A_{69} = instrument reading using filter #69

C = constant required to convert instrument readings to Hazen units, and is approximately equal to 1.35.

1.62 = turbidity - particulate slope correction
Multiply by the dilution factor if necessary.

NOTE: This method is applicable only when color does not exceed 100 H.U. or $A_{42} \leq 70$ and $A_{69} \leq 20$.

7. Precision and Accuracy

Routine samples analyzed in duplicate have a mean standard deviation of approximately 1 H.U.

Regression analysis indicates that correlation between this method and Method B - Visual Comparison is 97.6% for domestic water samples and 93.4% for river and lake samples.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1976). Standard Methods for the Examination of Water and Wastewater. 14th ed. APHA, Washington, D.C. 64-71.

- 8.2. Crowther, J. and Evans, J. (1977). Estimating Colour in Hazen Units by Spectrophotometer. Ministry of the Environment, Rexdale, Ontario. 19 pp.
- 8.3. Klett Manufacturing Company Inc. Industrial Manual, Klett-Summerson Photoelectric Colorimeter. New York, N.Y.

APPARENT COLOR

Visual Comparison Method B

SUMMARY

Matrix.	This method is used for color determinations in the field or when the apparatus required for Method A is unavailable.
Substance determined.	Apparent color in Color Units (also called Hazen Units).
Interpretation of results.	Color is a physical measurement which considers the combined effect of all colored components in the water. The most prevalent substances which contribute to natural color include humic acids, iron and manganese. The test is not generally applicable to industrial waste samples.
Principle of method.	An aliquot of sample is visually compared with a set of standardized glass color discs calibrated in Color Units (C.U.).
Time required for analysis.	Approximately 2 minutes are required for a single analysis. On a routine basis about 100 tests can be performed in a day.
Range of application.	Color is reported in the range from 5 to 70 Color Units. Laboratories possessing a set of discs for the range 70 to 250 Color Units should check the accuracy of the discs by comparison with prepared chloroplatinate standards. If verified, these discs can be used to extend the measuring range as long as the samples are of a normal matching hue.
Standard deviation.	5 C.U. in the 5 - 20 C.U. range 10 C.U. in the 20 - 70 C.U. range 15 C.U. in the 70 - 100 C.U. range 25 C.U. in the 100 - 250 C.U. range
Accuracy.	Not determined.
Limit of detection.	5 C.U.
Interferences and shortcomings.	The color of a sample is affected by changes in pH, biochemical activity, and by the precipitation of iron and manganese. The method is not applicable to colors other than those of natural water.

**Minimum volume
of sample.** 75 ml.

**Preservation and
sample container.** To minimize the processes which affect color, sample containers should be filled to capacity, sealed tightly and refrigerated until the test is performed. Immediate delivery to the laboratory is essential. Glass containers are preferred.

**Safety
considerations.** None.

APPARENT COLOR

Visual Comparison Method B

1. Introduction

Using matched Nessler tubes, the apparent color of the well mixed unfiltered sample is determined in an optical instrument (Lovibond Nesslerizer) by visual comparison with a graded series of colored glass discs calibrated in Hazen Units, using a standardized light source. Distilled water in a second Nessler tube placed under the discs assists the comparison by compensating for the depth of sample water viewed.

2. Interferences and Shortcomings

The color of a sample may be affected by a variety of chemical and biological phenomena which include changes in pH, biochemical oxidation or reduction and the precipitation of iron and manganese compounds. Proper handling and preservation prior to analysis will minimize these effects.

The available series of standard discs ranging from 70 to 250 Hazen Units was not designed specifically for the evaluation of water samples. Before use, their calibration should be verified by comparison with chloroplatinate standard solutions.

3. Apparatus

- 3.1. BDH Lovibond Nesslerizer with a white light cabinet.
- 3.2. BDH color discs calibrated in Hazen Units (Color Units).
- 3.3. Standardized light source, BDH bulb type 779.
- 3.4. Nessler tubes, 50 ml, tall.

4. Reagents

For calibration of the glass discs see Reagents 4.1 - 4.5 Method A.

5. Procedure

5.1. Calibration of Standard Discs

PRIOR TO USE OF GLASS DISCS IN THE FIELD A CALIBRATION CHECK IS NECESSARY.

- 5.1.1. Fill a 50 ml Nessler tube with standard.
- 5.1.2. Fill a 50 ml Nessler tube with distilled water.

- 5.1.3. Be sure glass surfaces of the Nessler tubes are wiped clean.
- 5.1.4. Place standard in sample side of Nesslerizer and distilled water tube in reference side.
- 5.1.5. Match color of the sample with color of the tube of clear water plus calibrated glass disc when viewed by looking toward a white surface. Glass discs are usually in agreement with platinum cobalt standards, their use is recognized as a standard field procedure (8.1).

5.2. Determination of Sample Color

- 5.2.1. Fill a 50 ml Nessler tube to the calibration line with sample.
- 5.2.2. Fill a second 50 ml Nessler tube to the calibration line with distilled water.
- 5.2.3. Be sure the glass surfaces of the Nessler tubes are wiped clean.
- 5.2.4. Place the sample in the sample side of the Nesslerizer and the distilled water tube in the reference side.
- 5.2.5. While viewing the tubes through the eye-piece of the Nesslerizer, systematically rotate discs of increasing depth of color into place above the reference tube until the color most closely matches that of the sample. A methodical technique improves reproducibility.
- 5.2.6. Record the value of the colored disc which best matches the color of the sample.

6. Calculation and Reporting

The recorded value of the colored disc is equivalent to the color of the sample in Color Units (C.U.).

Report to:

- 5 C.U. in the 5 - 20 C.U. range
- 10 C.U. in the 20 - 70 C.U. range
- 15 C.U. in the 70 - 100 C.U. range
- 25 C.U. in the 100 - 250 C.U. range

7. Precision and Accuracy

The standard deviation is dependent on the amount of color in the sample. Four standard deviations are defined for four ranges: 5 C.U. from 5 to 20 C.U., 10 C.U. from 20 - 70 C.U., 15 C.U. from 70 - 100 C.U., and 25 C.U. from 100 - 250 C.U.

Accuracy depends upon the proper calibration of the glass color discs using the platinum-cobalt method. Since these discs meet APHA specifications, the accuracy is within the precision of the method.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association, Water Pollution Control Federation (1975). 14th Edition. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, D.C., 64-71.

THE DETERMINATION OF CONDUCTIVITY

Conductivity is a measure of the capacity of a liquid to convey an electric current. This capacity is related to the total concentration of ionized substances in the water and is temperature dependent. The test is so precise and accurate that conductivity is frequently preferred to a dissolved solids test as an indicator of the dissolved solids content of a routine river or water sample.

Any aqueous system containing dissociated molecules will conduct an electric current. The positive ions migrate to the negative electrode (cathode), and the negative ions migrate to the positive electrode (anode). Most inorganic acids, bases and salts are good conductors, while molecules of most organic compounds do not dissociate in aqueous solution and are normally poor conductors. Most raw and finished waters in Canada and the United States have conductivities in the range of 50 - 500 micromhos/cm with highly mineralized waters having conductivities of over 1000 micromhos/cm.

Conductivity may also be measured on soil, sediment, industrial waste and mine tailing extracts. The conductivity measurement of a soil saturation extract or of other soil:water ratios is a useful indicator of salinity which in turn affects plant growth. In sediment studies, measurements of conductivity and other parameters are useful indicators of sediment-water interchanges. Conductivity (and pH) provide useful information regarding the quality of leachate in the field and column leach tests, and the "inertness" or otherwise of an industrial waste or "clean fill".

Sample Handling and Preservation

Water and Industrial Waste

Samples may be collected in glass or polyethylene containers. Preservatives must not be added to samples scheduled for conductivity determination.

Soil and Sediment

Samples may be collected in glass or plastic containers. No preservatives are used.

Selection of Method

For water samples a Radiometer CDM3 conductivity meter with a CDC 324 jacketed cell is used (Method A). This instrument affords precise, rapid, and simply performed measurements. Conductivity may also be estimated by a calculation based on the concentrations of the major ions present in the sample.

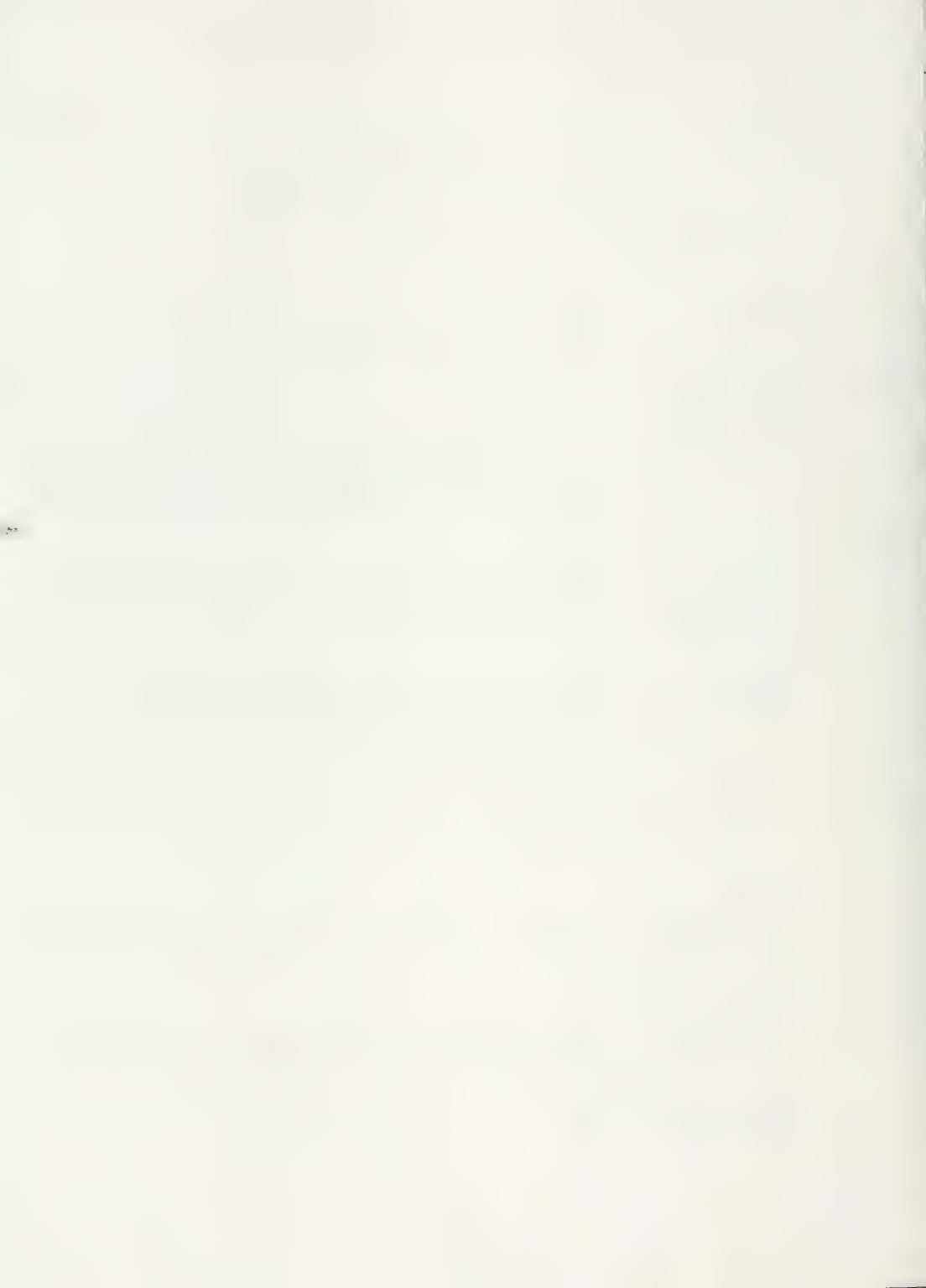
On soil, sediment and industrial waste samples, conductivity is measured using an Electronic Switchgear conductivity meter (Method B) when only a small volume of extract is available and when precise measurement at higher conductivity levels is not essential. For larger volumes of extract, the Radiometer (Method A) can be used. The conductivity of soils is usually measured on a saturation extract to approximate the salinity of the natural soil solution. Measurements are also made on interstitial water of sediments and on leachates and water:sample ratios of industrial waste or mine tailings.

CONDUCTIVITY

Radiometer Conductivity Meter Method A

SUMMARY

Matrix.	This method is used routinely on drinking and surface waters precipitation, sewage and industrial waste.
Substance determined.	The capacity of a water to carry an electric current.
Interpretation of results.	Results are reported in $\mu\text{mhos/cm}$ at 25°C . The conductivity may be used as an estimate of the total dissolved solids content of a water by multiplying the conductivity by a factor, usually between 0.55 and 0.90, which is specific for each water body. This approximation is valid only when the pH of the water lies between 5 and 9.
Principle of method.	The sample is introduced into a jacketed conductivity cell and equilibrated to 25°C . The conductivity is read on a suitable meter.
Time required for analysis.	Each determination requires about 1 minute after initial instrument set-up.
Range of application.	The conductivity ranges utilized are: precipitation: 0-150 $\mu\text{mhos/cm}$; surface water: 0-1500 $\mu\text{mhos/cm}$; drinking water: 0 - 1500 $\mu\text{mhos/cm}$; sewage and industrial waste: 0-2000 $\mu\text{mhos/cm}$.
Standard deviation.	± 0.185 to ± 8.66 $\mu\text{mhos/cm}$ depending on sample type and concentration (see Section 7).
Accuracy.	Recoveries of two Quality Control standards were 100% and 101% respectively.
Detection criteria.	0.304 $\mu\text{mhos/cm}$.
Interferences and shortcomings.	Air bubbles or foreign material trapped in the cell can lead to low results.
Minimum volume of sample.	70 ml.
Preservation and sample container.	Glass or polyethylene bottles are acceptable. Preservatives must not be used.
Safety considerations.	None.



CONDUCTIVITY

Radiometer Conductivity Meter Method A

1. Introduction

A Radiometer CDM3 conductivity meter and a CDC 324 jacketed conductivity cell are used to determine conductivity. A sample is aspirated by suction into the cell which is maintained at 25°C by a circulating water bath. The meter has a compensating circuit for the cell factor. The reading is multiplied by the appropriate power of ten to give conductivity in micromhos/cm.

2. Interferences and Shortcomings

The small capacity of the cell (approx. 1 cm³) renders it very sensitive to contamination by fibrous material. Such contamination causes low readings, and is difficult to remove except by backflushing the cell. Samples containing large quantities of particulate or fibrous suspended solids should be filtered before being introduced into the cell.

Air bubbles in the cell, caused by too great a sample aspiration rate, can also lead to low readings.

3. Apparatus

- 3.1. Conductivity meter, Radiometer CDM3.
- 3.2. Conductivity cell, jacketed, Radiometer CDC 324.
- 3.3. Circulating water bath, capable of maintaining 25.2°C ± 0.01°C (eg. Lauda type K2).
- 3.4. Beakers, 50 ml.

4. Reagents

- 4.1. Potassium chloride (KCl) reagent grade crystals.

4.2. Potassium Chloride Stock Solutions

4.2.1. Surface Water (0.1M KCl)

Dissolve 7.456 g potassium chloride (dried for 1 hour at 105°C) in distilled, deionized water. Dilute to 1 liter with distilled, deionized water.

4.2.2. Precipitation (0.01M KCl)

Dissolve 0.7456 g potassium chloride (dried for 1 hour at 105°C) in distilled, deionized water. Dilute to 1 liter with distilled, deionized water.

4.2.3. Drinking Water(0.132M KCl)

Dissolve 2.4599 g potassium chloride (dried for 1 hour at 140°C) in distilled, deionized water. Dilute to 250 ml with distilled, deionized water.

4.2.4. Sewage and Industrial Waste (1.0M KCl)

Dissolve 149.12 g potassium chloride (dried for 1 hour at 105°C) in distilled, deionized water. Dilute to 2 liters with distilled, deionized water.

4.3. Calibration Standard

A standard resistor is used for calibration and consists of a $2000 \Omega \pm 0.1\%$ metal film resistor mounted in an Amphenol 83 - ISP coaxial connector. Measurements should be within 1560 - 1600 $\mu\text{mhos/cm}$ with the meter in the 0.316 Cell Constant mode.

4.4. Quality Control Solutions

4.4.1. Surface Water (0-1500 $\mu\text{mhos/cm}$ range)

QC-A: Tap water with a conductivity of approximately 330 $\mu\text{mhos/cm}$ at 25°C is used as a QC-A.

QC-B: A 1:1 ratio of tap water to distilled water is used to give a conductivity of approximately 174 $\mu\text{mhos/cm}$ at 25°C.

Calibration Standard C: Dilute 40 ml stock solution to 4 liters to give a 0.001M solution with a conductivity of 147.0 $\mu\text{mhos/cm}$ at 25°C.

Calibration Standard D: Dilute 200 ml stock solution to 4 liters to give a 0.005M solution with a conductivity of 717.8 $\mu\text{mhos/cm}$ at 25°C.

4.4.2. Precipitation (0 - 150 $\mu\text{mhos/cm}$ range)

QC-A: Dilute 50 ml stock solution to 1 liter with distilled, deionized water. This 0.0005M solution has a conductivity of approximately 73.90 $\mu\text{mhos/cm}$ at 25°C.

QC-B: Dilute 10 ml stock solution to 1 liter with distilled, deionized water to give a 0.0001M solution with a conductivity of approximately 14.95 $\mu\text{mhos/cm}$ at 25°C.

4.4.3. Drinking Water (0 - 1500 $\mu\text{mhos/cm}$ range)

QC-A: Dilute 150 ml stock solution to 2 liters with distilled, deionized water. This gives a 0.0099M solution with a conductivity of 1399 $\mu\text{mhos/cm}$ on the 1500 $\mu\text{mhos/cm}$ scale at 25°C.

QC-B: Dilute 50 ml stock solution to 2 liters with distilled, deionized water to give a 0.00330M solution with a conductivity of 477.4 $\mu\text{mhos/cm}$ on the 500 $\mu\text{mhos/cm}$ scale at 25°C.

QC-C: Same as QC-B.

QC-D: Dilute 20 ml stock solution to 2 liters with distilled, deionized water to give a 0.00132M solution with a conductivity of 193.4 $\mu\text{mhos/cm}$ at 25°C.

NOTE: QC-A and QC-B are read on the 1500 scale and QC-C and QC-D are read on the 500 scale.

4.4.4. Sewage and Industrial Waste

QC-A: Dilute 20 ml stock solution to 2 liters with distilled, deionized water to give a 0.01M solution with a conductivity of 1413 $\mu\text{mhos/cm}$ at 25°C.

QC-B: Dilute 10 ml stock solution to 2 liters with distilled, deionized water to give a 0.005M solution with a conductivity of 717.8 $\mu\text{mhos/cm}$ at 25°C.

- 4.4.5. Occasionally samples will have conductivity values greater than 2000 $\mu\text{mhos/cm}$. For these cases, 2 higher standards are also prepared.

Potassium Chloride (1M)

Dissolve 74.56 g potassium chloride (dried for 1 hour at 105°C) in distilled, deionized water and dilute to 1 liter. This solution has a conductivity of 111,900 $\mu\text{mhos/cm}$ at 25°C and is read on the 150,000 scale.

Potassium Chloride (0.02M)

Dilute 20 ml of 1M potassium chloride solution to 1 liter with distilled, deionized water. This solution has a conductivity of 2767 $\mu\text{mhos/cm}$ at 25°C and is read on the 5000 scale.

5. Procedure

5.1. Calibration

With the exception of surface water analysis, the conductivity meter is calibrated with a standard resistor. Surface waters are calibrated using calibration standards as in 5.3.3 and 5.3.4.

- 5.1.1. Disconnect cell and connect standard resistor.
- 5.1.2. Set METER RANGE = 1500 $\mu\text{mhos/cm}$
 CELL CONSTANT CORRECTION = 0.0%
- 5.1.3. Record the reading from the 1500 $\mu\text{mhos/cm}$ scale. The value must lie between 1560 and 1600 $\mu\text{mhos/cm}$.
- 5.1.4. Reconnect the conductivity cell.

5.2. Instrument Set-up

- 5.2.1. Turn on the cooling water to the circulating water bath. A continuous slow flow of water should be sufficient to reduce the bath temperature below 25°C as indicated by the control thermometer. Check the water level in the water bath and top up with distilled water as required.
- 5.2.2. Switch on the circulating water bath. The pump light and heater light should come on.

- 5.2.3. Adjust the pump flow control for full flow through the conductivity cell jacket.
- 5.2.4. Set the PROPORTIONING control to 8.
- 5.2.5. Allow 5 minutes for the bath temperature to stabilize. If at the end of this time the bath temperature is not 25.2 °C, adjust the thermostat.

NOTE: Do not attempt to adjust the regulator without loosening the set screw.

The control thermometer will read differently from the reference thermometer. The reference thermometer reading is taken as the true water bath temperature. The temperature in the cell will be 0.2 °C lower than that in the bath.

The heater light should be turning on and off every few seconds. If it is on a majority of the time once the bath is at the required temperature, the flow of cooling water should be reduced or the heater input rheostat setting should be raised.

- 5.2.6. Switch on the conductivity meter.
- 5.2.7. Turn on the aspirator tap to apply moderate suction to the conductivity cell.

NOTE: Too strong a suction will induce air bubbles in the cell and result in low readings.

5.3. Conductivity Measurements

- 5.3.1. Set the CELL CONSTANT CORRECTION % to the value stamped on the cell head. Unless analyzing surface waters, skip step 5.3.3.
- 5.3.2. Flush cell with distilled water.
- 5.3.3. Calibrate meter using calibration standards and the instructions in the instrument manual.
- 5.3.4. Read and record QC-A and QC-B.
- 5.3.5. Flush the cell with distilled water until the meter yields a steady reading between 1 and 2 µmhos/cm on the 0-5 scale.

- 5.3.6. Flush and fill the cell with sample, allow to equilibrate to 25°C, and select the meter range giving the maximum on-scale pointer deflection. Read the conductivity and record the conductivity to 3 significant figures. Record meter range. Check that the reading is appropriate for that particular range.
- 5.3.7. Aspirate an additional amount of the same sample into the cell. If the reading changes by more than 5%, repeat step 5.3.6. Such changes will be noticeable when successive sample conductivity values differ by a factor of 5 or greater.
- 5.3.8. After every 10 samples, and at the end of the day's work, flush the cell with distilled water until the conductivity reads between 1 and 2 $\mu\text{mhos/cm}$.

6. Calculation and Reporting

Report the conductivity in $\mu\text{mhos/cm}$ at 25°C according to the following:

Conductivity less than 100 $\mu\text{mhos/cm}$ report 2 significant figures.

Conductivity 100 $\mu\text{mhos/cm}$ or greater - report 3 significant figures.

7. Precision and Accuracy

Sample	Concentration Range ($\mu\text{mhos/cm}$)	S_{ld}	S_{md}	S_{hd}
Precipitation	0-150	0.185	-	-
Surface Water	0-50	0.22	0.39	0.47
	0-150	0.48	1.15	1.06
	0-500	1.37	1.96	1.80
	0-1500	4.98	5.46	8.66
Drinking Water	0-1500	1.66	2.07	6.85
Sewage and Industrial Waste	0-2000	1.70	2.34	4.29

Where:

S_{ld} = standard deviation low level within-run duplicates at 0-20% of operating range.

S_{md} = standard deviation mid-level within-run duplicates at 20-50% of operating range.

S_{hd} = standard deviation high-level within-run duplicates at 50-100% of operating range.

Recoveries of two Quality Control standards were 100% and 101% respectively.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1980). Standard Methods for the Examination of Water and Wastewater. 15th ed. APHA, Washington, D.C., 70-73.
- 8.2. Instruction Manual; Radiometer Conductivity Meter CDM 3.
- 8.3. U.S. Geological Survey (1970). Specific Conductance as a Means of Estimating Ionic Strength. Geological Survey Professional Paper 700-D. U.S. Department of the Interior, D-272-D-280.

CONDUCTIVITY

Electronic Switchgear Conductivity Meter Method B

SUMMARY

Matrix.	This method is used on soil, sediment, and industrial waste extracts when only small volumes of extract are available and when precise measurements at high conductivity levels are not essential.
Substance determined.	The capacity of a solution to carry an electric current.
Interpretation of results.	Results are reported as $\mu\text{mhos/cm}$ at 25°C . Conductivity may be used to provide information on salinity measurements of soil solutions, leachate quality and the inertness of industrial waste or "clean fill".
Principle of method.	The sample extract is introduced into a jacketed conductivity cell and the conductivity read on a suitable meter. The measured electrical resistance is a function of the electrolyte concentration and hence salt concentration of the solution.
Time required for analysis.	Preparation time varies with sample type and preparation method used. The actual conductivity measurement only requires about 1 minute per sample.
Range of application.	The conductivity range utilized is 1 - 10,000 $\mu\text{mhos/cm}$.
Standard deviation.	Not yet established.
Accuracy.	Not yet established.
Detection criteria.	Depends on cell used.
Interferences and shortcomings.	Air bubbles or foreign material trapped in the cell can lead to low results.
Minimum volume of sample.	Varies with sample preparation used. 50 g is recommended.
Preservation and sample container.	Soil samples may be collected in pomade jar or polyethylene bags and are air dried when returned to the laboratory. Industrial waste and sediment may be collected in glass or plastic bottles. No preservatives are used.
Safety considerations.	None.

CONDUCTIVITY

Electronic Switchgear Conductivity Meter Method B

1. Introduction

Conductivity measurements are made on soil-water pastes, 1:1 and 1:2 soil:water ratios, 1:5 industrial waste:water ratios, interstitial water of soils and sediments and column leachates of soil, sediments and industrial wastes. The conductivity is measured by an Electronic Switchgear Conductivity Meter with a conductivity cell having a cell constant of 1.0.

2. Interferences and Shortcomings

Fibrous material may contaminate the cell and lead to low readings.

3. Apparatus

- 3.1. Conductivity meter, Electronic Switchgear Ltd. Model MC-1 Mark V.
- 3.2. Conductivity cells, with cell constants $K = 1.0$ and $K = 0.1$.
- 3.3. Mechanical vacuum extractor, Concept Engineering recommended.
- 3.4. Suction assembly.
- 3.5. Buchner flasks.
- 3.6. Filter paper, fine.

4. Reagents

- 4.1. Potassium chloride (KCl), reagent grade crystals.

4.2. Potassium Chloride Solution (0.005M)

Dissolve 0.7456 g potassium chloride (oven dried for 1 hour at 109°C) in distilled water and dilute to 2 liters. The conductivity of this solution is $717.8 \mu\text{ mhos/cm}$ at 25°C .

4.3. Potassium Chloride Solution (0.001M)

Dilute 200 ml of 0.005M potassium chloride solution to 1 liter with distilled water. The conductivity of this solution is $147 \mu\text{ mhos/cm}$ at 25°C .

5. Procedure

5.1. Preparation of Saturation Extracts of Soils

- 5.1.1. Place 20-50 g air dried soil in a 250 ml beaker.

- 5.1.2. Using a pipette or burette, add distilled water and stir mixture with spatula until soil is saturated. Consolidate mixture by tapping container on bench periodically during mixing. When saturated, soil paste glistens, flows slightly when container is tipped and slides freely off spatula (with exception of soils with a high clay content).

NOTE: Dry organic soils require an overnight wetting period as the pastes of such samples stiffen on standing and require the addition of water and remixing.

Course textured soils are readily saturated. Add additional soil if free water stands on surface after equilibration.

To fine textured soil add sufficient water immediately with little stirring to prevent puddling.

- 5.1.3. Cover beaker with watch glass and allow samples to stand for at least 1 hour. Recheck criteria for saturation. Add water or soil as necessary and remix.

- 5.1.4. Record volume of water added and weight of soil used and calculate percentage saturation as total weight of water in sample (added water and hygroscopic water) as a percentage of the oven dry weight of soil. Alternatively, calculate saturation percentage by oven drying a weighed subsample of the saturated paste at 105°C and reweighing.

- 5.1.5. Allow saturated paste to stand 4 hours or more. Transfer to a Buchner funnel or to the suction assembly, both fitted with low ash, highly retentive filter paper. Apply vacuum and collect filtrate (at least 25 ml). Determine conductivity on filtrate according to 5.5.

5.2. **Preparation of a Sample:Water Ratio for Soil, Sediment, Industrial Waste and Mine Tailings**

- 5.2.1. For a 1:1 sample:water ratio, weigh 25 g air dried sample into an Erlenmeyer flask and add 25 ml distilled water. For a 1:2 sample:water ratio, weigh 15 g air dried sample and add 30 ml distilled water. For a 1:5 sample:water ratio, weigh 6 g air dried sample and add 30 ml distilled water. The 1:2 or 1:5 ratios may be required for samples with a high absorptive capacity for water. Record ratio used.

NOTE: Weights of soil are arbitrary but soil:water ratio should be maintained at 1:1, 1:2 or 1:5.

- 5.2.2. Shake mixture for 30 minutes on a reciprocating shaker.
- 5.2.3. Measure conductivity of filtrate according to 5.5, using a cell with cell constant $K = 1.0$.

5.3. Preparation of Sediment for Conductivity Measurement of Interstitial Water

- 5.3.1. Pour off free water above sediment. Place wet sediment on several filter paper discs and allow to drain. Gently squeeze sample between filter paper to remove the remainder of the free water.
- 5.3.2. Place at least 50 g damp sediment into suction assembly used in 5.1 and apply vacuum as for Saturation Extract using mechanical vacuum extractor.
- 5.3.3. Determine conductivity on extract according to 5.5.

5.4. Preparation of Column Leachates

- 5.4.1. Collect leachate using normal procedure.
- 5.4.2. Measure conductivity according to 5.5. if only a small amount of leachate is available (25 ml). For larger volumes measure conductivity according to Method A.

5.5. Conductivity Measurement

NOTE: Conductivity and pH may be done on "cuts" of column leachate since a combination electrode can also be placed in the cell.

- 5.5.1. Check that the conductivity meter is operating accurately by measuring and recording the conductivity of the 0.005 M and 0.001 M potassium chloride solution. Replace battery if required.
- 5.5.2. Transfer sample liquid to one of the two cells. If conductivity is expected to be high use the smaller of the cells ($K = 1.0$).
- 5.5.3. Measure and adjust for temperature. Hold ACTIVATOR switch down and search for appropriate range using RANGE switch. Manipulate dial to give "null" balance as shown by the indicator.
- 5.5.4. Note RANGE setting and factor for cell used, and dial reading. If the conductivity is low and if at least 25 ml is available, repeat test using 0.1 K cell.
- 5.5.5. Calculate conductivity using scale factor for the appropriate cell.

6. Calculation and Reporting

Read conductivity and calculate using scale factor for appropriate cell. Report conductivity in $\mu\text{mhos/cm}$ at 25°C to 2 significant figures.

7. Precision and Accuracy

Not yet determined.

8. Bibliography

- 8.1. Canada Soil Survey Committee (1978). Manual on Soil Sampling and Methods of Analysis. J. A. McKeague (ed.) Canadian Society of Soil Science, Ottawa, Ontario, 69-70.

THE DETERMINATION OF CYANIDE

Cyanide is one of the simplest and most readily formed organic molecules and its derivatives are almost universally present in the environment. Cyanides exist as metabolic intermediates in plants and animals and are very important in a number of industrial processes.

In Ontario, air and water borne effluents from steel manufacturers are major contributors of cyanide in the aquatic environment. Waste water from precious metal mining operations and metal plating industries also contain significant amounts of cyanide and metal cyanide complexes. Calcium cyanamide is used as a herbicide, as a cotton defoliant and as a pesticide.

The most significant environmental effect of cyanide is its toxicity to fish and concentrations of between 50 and 180 $\mu\text{g/l}$ may be lethal. Continuous flow bioassays yield somewhat lower results and reduced growth rates, diminished fecundity and mortality were noted at cyanide levels as low as 18 $\mu\text{g/l}$. Humans are somewhat less sensitive to cyanide, however, toxic effects include nausea, vomiting and eventual death.

Cyanide is environmentally significant in a number of forms. Complex cyanides, such as $(\text{Fe}(\text{CN})_6)^{3-}$, and $(\text{Fe}(\text{CN})_6)^{4-}$ can decompose to form free cyanide and are therefore important in terms of pollution potential. Although thiocyanate is more stable to decomposition than the complex cyanides, under ultraviolet radiation it may also degrade to free cyanide, resulting in a potential pollution source.

The simple cyanide ion, CN^- , and hydrocyanic acid, HCN , are the forms most toxic to fish and consequently are most important for the establishment of a cyanide criterion. Currently, the maximum permissible level of cyanide in public water supplies is 0.01 mg/l with a water quality objective of 0.005 mg/l.

Sample Handling and Preservation

Water, Sewage, Industrial Waste and Landfill Leachates

A minimum sample of 1 liter is preferred for cyanide analysis. Glass or plastic bottles are satisfactory. Since cyanide is perishable, samples must be preserved by the addition of sodium hydroxide to a pH greater than 11. Preserved samples are stored in a cool, dark place if possible. Samples stored under these conditions are stable for at least one week. Samples containing oxidants such as chlorine should be treated with sodium arsenite or oxalic acid to remove the oxidant prior to sodium hydroxide preservation. Samples containing sulphide may be treated with lead carbonate and filtered to remove the precipitated lead sulphide prior to alkaline preservation.

Soils and Sediments

Samples are collected in wide mouth glass or plastic jars, air dried and ground to a uniform particle size. An aqueous extract, normally 1:5 or 1:10 soil to distilled water, is then prepared for cyanide determination.

Vegetation

Samples are collected in perforated polyethylene bags, air or oven dried, ground to a uniform particle size and stored in glass jars. An aqueous extract is prepared for cyanide determination.

Selection of Method

Procedures available for cyanide determination include manual and automated colorimetric, electrochemical, titrimetric, gas chromatographic and specific ion electrode procedures. The specific ion electrode is limited by a lack of low level sensitivity and is subject to interferences. Gas chromatographic procedures are sensitive but time consuming. Titrimetry is rapid and simple for high level samples but lacks low level sensitivity. Electrochemical procedures are relatively interference free and have fairly good low level sensitivities. Colorimetric procedures, employing the pyridine-barbituric acid reagent, are rapid, rugged and have the best low level sensitivities of all the methods. The method, however, is subject to interferences which must be removed by sample pretreatment. Currently an automated colorimetric procedure employing an automated distillation pretreatment step is used to screen all samples for the presence of cyanide or cyanide precursors. Method A, this procedure, however, does not remove thiocyanate interferences. All prescreened samples exhibiting cyanide levels greater than 0.01 mg/l are manually distilled and reanalyzed with the automated colorimetric procedure to determine total cyanide.

Free cyanide is determined by employing a gas dialysis separation step as a pretreatment prior to colorimetric analysis. Method B, this procedure is interference free so that results may be reported without further treatment.

TOTAL CYANIDE

Manual Distillation - Automated Colorimetric Method A

SUMMARY

Matrix.	This method is applicable to waters, sewages, trade wastes, landfill leachates and aqueous extracts of soil and vegetation.
Substance determined.	Total cyanide, which includes the cyanide ion derived from HCN and simple cyanides such as KCN as well as those derived from complex cyanide ions such as ferri and ferro cyanide, after acidic digestion and distillation.
Interpretation of results.	Results are reported as mg/l as CN.
Principle of method.	Free and complexed cyanides are manually distilled as HCN, from a solution containing excess tartaric acid. The distillate is collected as a 0.01N aqueous sodium hydroxide solution. Cyanide is determined by automated colorimetry by reaction with chloramine-T to form cyanogen chloride which reacts with barbituric acid in pyridine to form a red colored compound, the intensity of which is measured at 580 nm.
Time required for analysis.	Samples are prescreened for the presence of cyanide at the rate of 20 samples per hour. Approximately 16 samples can be manually distilled and analyzed for total cyanide in one day.
Range of application.	The working range for the automated colorimetric procedure is 0.005 to 0.5 mg/l as CN. The range is extended by sample dilution. The size of the aliquot taken for manual distillation is selected on the basis of the results from prescreening in order to obtain a mid scale reading on the distillate.
Standard deviation.	The automated colorimetric procedure is repeatable to within 0.005 mg/l as CN over the range of calibration, based upon duplicate samples. The manual distillation procedure is repeatable to within approximately 10%.
Accuracy.	The accuracy of the manual distillation procedure is ± 10 RSD based upon replicate distillations of complexed cyanide solutions.
Detection criteria	.022 mg/l.

**Interferences
and shortcomings.**

Thiocyanate, which interferes in the direct colorimetric determination of cyanide is removed in the distillation process. Steam distillable organic interferences may be removed by solvent extraction prior to distillation. Sulphides are removed by the addition of lead carbonate to the distillation flask. Oxidizing agents, such as chlorine, which destroy the free cyanide fraction, must be removed at the time the sample is taken prior to preservation with sodium hydroxide.

**Minimum volume
of sample.**

A minimum of 500 ml is required for a detection limit of 0.01 mg/l as CN, however a 1 liter sample is preferred. For soils and vegetation, sufficient sample is required to allow for a 1 g to 5 g representative sample to be taken.

**Preservation and
sample container.**

Glass or plastic bottles are suitable. Samples are preserved with sodium hydroxide to a pH greater than 11. If free cyanide is to be determined separately, the samples should be kept cool and in the dark to prevent degradation of complexed cyanides. Oxidants, such as chlorine, the presence of which are indicated by formation of a blue color on a starch-iodide test paper, are removed by the addition of either sodium arsenite (0.1mg/l) or oxalic acid (2 g/l) prior to preservation with sodium hydroxide.

**Safety
considerations.**

Cyanide is extremely toxic. Samples suspected of containing high cyanide concentrations should be marked accordingly. Potassium cyanide standards and reagents must be handled with care to prevent skin contact or ingestion. Pyridine should be dispensed carefully in a fume hood to prevent inhalation and skin contact should be avoided.

TOTAL CYANIDE

Manual Distillation Automated Colorimetric Method A

1. Introduction

The cyanide ion, CN^- and complexed metal cyanides such as ferrocyanide ($\text{Fe}(\text{CN})_6^{4-}$) and cuprous cyanide (CuCN) are converted to HCN by boiling in the presence of excess tartaric acid. The HCN is distilled into 0.01N sodium hydroxide where it is trapped as CN^- .

In an automated analysis procedure, the pH of the distillate is adjusted to 5.2 and reacted with chloramine-T, forming cyanogen chloride, ClCN . The cyanogen chloride further reacts with barbituric acid in pyridine to form a colored complex which is measured spectrophotometrically at 580 nm.

2. Interferences and Shortcomings

Common interferences in the colorimetric analysis for cyanide include: sulphide, heavy metal cations, fatty acids and other steam-distillable organics; substances which may hydrolyze to form cyanide under acidic distillation, such as thiourea and cysteine; substances contributing to color or turbidity thereby affecting absorption of light in the flow cell; and oxidizing agents which may result in the destruction of cyanide either before or during the distillation stage.

Sulphide is removed by treating the alkaline sample at pH 11 with small increments of powdered lead carbonate. Black lead sulphide precipitates in samples containing sulphide. This operation is repeated until precipitation is complete. The precipitate is filtered and rinsed, adding the rinse water to the filtrate. To minimize complexing and occlusion of the cyanide with the precipitate, adding large excesses of lead carbonate and long periods of contact are avoided.

Steam distillable fatty acids and other organics may be removed by solvent extraction. The sample is acidified to pH 6.7 with acetic acid and extracted with a volume of isooctane, hexane or chloroform (in order of preference) equal to 20% of the sample volume. Multiple extractions or a long contact time at a low pH are avoided to minimize the loss of HCN .

Oxidizing agents such as chlorine are removed by the addition of sodium arsenite (0.1 g/l) or oxalic acid (2 g/l) until a starch-iodide test paper gives no response (no blue color formation).

All other common interferences are removed by the distillation procedure.

3. Apparatus

- 3.1. Round bottom side arm flasks, 1000 ml, with B24/40 ground glass joint.
- 3.2. Delivery tubes and water condensers (Fig. 1).
- 3.3. Meeker burners and asbestos gauze.

- 3.4. Measuring cylinders, 250 and 500 ml.
- 3.5. Automated colorimetric apparatus, including:
 - 3.5.1. Sampler.
 - 3.5.2. Technicon proportioning pump III.
 - 3.5.3. Technicon II colorimeter with 50 mm x 1.5 mm ID flow cells and 580 nm filters.
 - 3.5.4. Associated pump tubing manifold (Figure 2).
 - 3.5.5. Distillation bath with coil, variable temperature to at least 150 °C.
 - 3.5.6. Strip chart recorder.

4. Reagents

- 4.1. Sodium arsenite (NaAsO_2) reagent grade crystals.
- 4.2. Lead carbonate ($\text{PbCO}_3 \cdot \text{Pb(OH)}_2$) reagent grade.
- 4.3. Sodium hydroxide (NaOH) reagent grade pellets.
- 4.4. Methyl red indicator, reagent grade.
- 4.5. Barbituric acid ($\text{CH}_2\text{CO.NH.CO.NH.CO}$) BDH reagent.
- 4.6. Chloramine-T ($1\text{-CH}_3\text{C}_6\text{H}_4\text{-4-SO}_2\text{NClNa} \cdot 3\text{H}_2\text{O}$) reagent grade.
- 4.7. Pyridine ($\text{C}_5\text{H}_5\text{N}$) reagent grade.
- 4.8. Disodium hydrogen phosphate (Na_2HPO_4) reagent grade.
- 4.9. Potassium dihydrogen orthophosphate (KH_2PO_4) reagent grade.
- 4.10. Tartaric acid ($(\text{CHOH.COOH})_2$) reagent grade.
- 4.11. Potassium cyanide (KCN) reagent grade.
- 4.12. Phosphoric acid (H_3PO_4) 85% reagent grade.
- 4.13. Hypophosphorous acid (H_3PO_2) reagent grade.
- 4.14. Hydrochloric acid (HCl) reagent grade.
- 4.15. **Methyl Red Indicator Solution (0.1%)**
Dissolve 0.1 g methyl red in 100 ml of 60:40 methanol:water solvent.
- 4.16. **Sodium Hydroxide Solution (1.0N)**
Dissolve 40.0 g of sodium hydroxide pellets in 800 ml of distilled water, cool and make up to 1 liter in a volumetric flask.
- 4.17. **Tartaric Acid Solution (15%)**
Dissolve 150 g of tartaric acid in distilled water and make up to 1000 ml.
- 4.18. **Chloramine-T solution**
Dissolve 2.0 g of chloramine-T in distilled water and dilute to 500 ml.

4.19. Phosphate Buffer Solution (pH 5.2)

Dissolve 13.60 g of potassium dihydrogen orthophosphate and 0.28 g of disodium hydrogen phosphate in 900 ml of distilled water and make up to 1.0 liter in a volumetric flask.

4.20. Buffered Chloramine-T Solution

Add 200 ml of Chloramine-T solution to 800 ml of phosphate buffer and mix thoroughly.

4.21. Pyridine-Barbituric Acid Color Reagent

Place 15.0 g of barbituric acid into a 1 liter volumetric flask, add 100 ml of distilled water and mix to thoroughly wet and disperse lumps of barbituric acid. Add 15 ml of concentrated hydrochloric acid and mix well. Add 75 ml of pyridine and mix. Add 800 ml of distilled water and stir until the barbituric acid has dissolved. Dilute the contents of the flask to 1.0 liter with distilled water. Store in a cool, dark place.

NOTE: BARBITURIC ACID IS A RESTRICTED MATERIAL AND MUST BE STORED IN A LOCKED CABINET.

4.22. Distillation Acid

Carefully add 250 ml of 85% phosphoric acid to 500 ml of distilled water. Add 50 ml of hypophosphorous acid and mix well. Dilute to 1000 ml with distilled water.

4.23. Cyanide Stock Solution (100 mg/l as CN)

Dissolve 0.250 g of potassium cyanide in 600 ml of distilled water containing 100 ml of 1.0N sodium hydroxide solution. Dilute to 1.0 liter in a volumetric flask and mix thoroughly.

NOTE: CYANIDE IS EXTREMELY TOXIC AND MUST BE HANDLED WITH CARE TO AVOID SKIN CONTACT AND/OR INGESTION OF THE DRY POWDER OR CONCENTRATED SOLUTION.

4.24. Cyanide Intermediate Solution (10 mg/l as CN)

Pipette 100 ml of stock cyanide solution into a 1 liter volumetric flask. Add 90 ml of 1.0N sodium hydroxide solution and dilute to volume with distilled water.

4.25. Working Cyanide Standards

Dilute 10.0, 20.0, 30.0 and 40.0 ml aliquots of cyanide intermediate solution to 1 liter in volumetric flasks containing 9 ml of 1.0N sodium hydroxide to give working standards of 0.1, 0.2, 0.3 and 0.4 mg/l as CN in 0.01N sodium hydroxide. These standards are stable, provided they are capped when not in use and may be used for several weeks.

4.26. Quality Control Solutions

Since cyanide is a perishable parameter, quality control solutions are not stable for extended periods of time. Composite samples of at least one month's supply of distillate are judiciously blended to give solutions with cyanide concentrations of about 10% and 80% of the operating range of the automated colorimetric procedure. Alternatively, pure standards may be

prepared in a similar manner as quality control solutions. These solutions are stable for several weeks provided proper preservation procedures are followed.

5. Procedure

5.1. Automated Distillation - Colorimetric Procedure

All samples are run as described in this section as a prescreening procedure to determine which samples require manual distillation for total cyanide determination, (5.2). The cyanide levels found in the prescreening are used to determine the size of the aliquot taken for the manual distillation. The distillation removes interfering substances. Samples reading less than 0.01 mg/l as CN are reported as such.

- 5.1.1. Set up the manifold as shown in Figure 2.
- 5.1.2. Set the temperature of the heating bath to 150 °C.
- 5.1.3. After a 30 minute warm-up period, run a baseline with all reagents, feeding distilled water through the sample line.
- 5.1.4. Calibrate the system according to established AutoAnalyzer procedures using the working standards.
- 5.1.5. Run QC samples and compare results with previous data, ensuring correct response before proceeding with sample analysis.
- 5.1.6. Run samples in groups of 10, separated by at least one check standard.
- 5.1.7. Compare results of unknown samples to the standard curve and report as mg/l total cyanide.

5.2. Manual Distillation Procedure

- 5.2.1. Set up the distillation apparatus as shown in Figure 1. A normal run consists of 7 samples and 1 quality control sample.
- 5.2.2. The pH of a properly preserved sample will be greater than 11. Samples with a pH of less than 11 are not normally analyzed but are reported as improperly preserved.
- 5.2.3. To a side arm flask add a sample aliquot as determined from the results obtained in 5.1. The size of the aliquot should be such that the cyanide concentration of the distillate lies between 0.1 mg/l and 0.3 mg/l whenever possible. Add distilled water to a total volume of about 500 ml.
- 5.2.4. Add 1 g each of sodium arsenite and lead carbonate to the flask. If a black lead sulphide precipitate forms, add additional lead carbonate until no further precipitate forms. Filter the sample, wash the flask, and then reintroduce the aliquot filtrate. Avoid prolonged contact of the solution with the precipitate to minimize the loss due to complexation or occlusion of cyanide with the precipitate.

- 5.2.5. Add 12 drops of methyl red indicator and at least 30 ml of 15% tartaric acid solution so that the contents of the flask are acidic as indicated by a persistent pink color.
- 5.2.6. Place 50 ml of 1N sodium hydroxide into a Nessler tube and place the tube into a 250 ml measuring cylinder.
- 5.2.7. Connect the condenser and delivery tubes such that the distillate passes through the sodium hydroxide solution.
- 5.2.8. Ignite the Meeker burner, adjusting the heating rate to ensure a full rolling boil without solution carryover. Ensure that all joints are tight.
- 5.2.9. Distill the sample until the measuring cylinder contains about 220 ml of solution.
- 5.2.10. After extinguishing the flame, loosen the stopper and remove the delivery tube from the cylinder.
- 5.2.11. Remove the Nessler tube and carefully transfer the contents back into the measuring cylinder. Wash the tube inside and out, returning all washings to the measuring cylinder. Make up the volume to 250 ml with distilled water and mix well.
- 5.2.12. Analyze the collected distillate using the automated distillation-colorimetric procedure as described in 5.1.

6. Calculation and Reporting

Total cyanide concentrations are calculated as follows:

$$\text{mg/l cyanide} = \frac{(r \times 250 \times \text{DF})}{V}$$

Where:

r = chart reading in mg/l cyanide

DF = dilution factor

V = sample aliquot taken

Results are reported to 2 significant figures. Samples with measurements of less than 0.01 mg/l after the prescreening are reported as such and are not re-run.

7. Precision and Accuracy

The automated colorimetric procedure has a standard deviation of 0.005 mg/l as CN based upon replicate analysis of standards.

Precision of the distillation procedure is affected by variations in distillation rate, losses due to leaking joints and composition of the sample. Variability should be no greater than 10% based upon replicate distillations.

Accuracy of the test is determined by the relative amounts of the complexed cyanides present in the sample. Calibration is controlled by 2 independently prepared standards at 80% and 10% of range. (QC-A and QC-B) standards are prepared by judicious blending of at least 1 month's supply of distillate. These solutions are analyzed and their values controlled to within 10% of the expected values.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation. (1975). Standard Methods for the Examination of Water and Wastewater. 14th edition, APHA, Washington, D.C. 370-372.
- 8.2. Technicon AutoAnalyzer II Methodology. Industrial Method No. 119-71 W/Preliminary, Oct. 1972; No. 353-75 W/A, May 1976.
- 8.3. U.S. Environmental Protection Agency (1979). Methods for Chemical Analysis of Water and Wastes. Environmental Monitoring and Support Laboratory, EPA Environmental Research Centre, Cincinnati, Ohio. EPA-600/4-79-020.

FREE CYANIDE

Automated Gas Dialysis Colorimetric Method B

SUMMARY

Matrix.	This method is used for water, sewages, trade wastes, landfill leachates and aqueous extracts of soil and vegetation.
Substance determined.	Free or simple cyanide ion as derived from HCN or simple cyanides such as KCN and NaCN.
Principle of method.	Free cyanide, CN^- , is isolated from an acidified sample aliquot as HCN by gas dialysis. An alkaline absorber stream fixes the dialysate as CN^- which is then determined by automated colorimetry by reaction of buffered chloramine-T to form cyanogen chloride which reacts with barbituric acid in pyridine to form a red colored compound. The intensity of the color produced is measured at 580 nm.
Time required for analysis.	Samples are analyzed for free cyanide at the rate of 20 samples per hour using an automated colorimetric procedure.
Range of application.	The working range of the method is from 0.005 mg/l to 0.5 mg/l as CN. The range is extended by sample dilution.
Standard deviation.	The automated procedure is repeatable to within 0.005 mg/l as CN over the range of calibration.
Accuracy.	Accuracy is controlled by standard solutions with concentrations at 10% and 80% of the range. Free cyanide is a perishable and convertible parameter so that quality control solutions containing complexed cyanides are not stable.
Detection criteria.	.002 mg/l.
Interferences and shortcomings.	<p>All known interferences which do not form gaseous products in acidic solution are excluded by the gas dialysis membrane. Sulfide, as H_2S, does not interfere at levels of at least 50 mg/l.</p> <p>Complex cyanides may be converted to free cyanide upon exposure to heat and light, resulting in a higher reading for free cyanide. Oxidants such as chlorine which destroy the free cyanide must be removed prior to preservation.</p>

**Minimum volume
of sample.**

A minimum of 100 ml is required.

**Preservation and
sample container.**

Samples are preserved with sodium hydroxide to a pH greater than 11. Samples containing oxidants such as Cl_2 should be treated with either sodium arsenite (0.1 g/l) or oxalic acid (2 g/l) for 15 minutes prior to addition of the sodium hydroxide preservative. The presence of oxidants is indicated by the appearance of a dark bluish color on a potassium iodide-starch indicator paper when exposed to a drop of the sample. Decomposition of complex cyanides is reduced by keeping the sample cool and stored away from light.

Samples may be submitted in either glass or plastic bottles.

**Safety
considerations.**

Cyanide is extremely toxic. Samples suspected of containing high cyanide concentrations should be marked accordingly. Pyridine should be handled carefully under a fume hood to prevent inhalation and skin contact should be avoided.

FREE CYANIDE

Automated Gas Dialysis Colorimetric Method B

1. Introduction

The free cyanide ion, CN^- , is converted to HCN gas in a 10% phosphoric acid solution, isolated from non-gaseous components by diffusion through a gas dialysis membrane and absorbed into a 0.01N solution of sodium hydroxide. The pH of the absorbing solution is adjusted to 5.2 with buffered chloramine-T, forming cyanogen chloride, ClCN . The ClCN then reacts with barbituric acid in pyridine to form a colored complex which is measured spectrophotometrically at 580 nm.

2. Interferences and Shortcomings

All common non-gaseous interferences are excluded from the automated colorimetric procedure by the gas dialysis membrane. Sulphide, which may pass through the membrane as H_2S does not interfere at levels of at least 50 mg/l as S.

3. Apparatus

3.1. Automated colorimetric apparatus, including:

- 3.1.1. Sampler.
- 3.1.2. Technicon Proportioning Pump III.
- 3.1.3. Technicon II Colorimeter with 580 nm filters and 50 mm x 1.5 mm I.D. flow cell.
- 3.1.4. Associated pump tubing manifold (see Figure 3).
- 3.1.5. 6" dialyser blocks (2) with Type C and Teflon type membranes.
- 3.1.6. Strip chart recorder.

4. Reagents

- 4.1. Sodium hydroxide (NaOH) reagent grade pellets.
- 4.2. Barbituric acid ($\text{CH}_2\text{CO.NH.CO.NH.CO}$) BDH reagent grade.
- 4.3. Pyridine ($\text{C}_5\text{H}_5\text{N}$) reagent grade.
- 4.4. Chloramine-T ($1\text{-CH}_3\text{C}_6\text{H}_4\text{-4-SO}_2\text{NCINa.3H}_2\text{O}$) reagent grade.
- 4.5. Phosphoric acid (H_3PO_4) 85% reagent grade.
- 4.6. Hypophosphorous acid (H_3PO_2) reagent grade.
- 4.7. Disodium hydrogen phosphate (Na_2HPO_4) reagent grade.
- 4.8. Potassium dihydrogen orthophosphate (KH_2PO_4) reagent grade.

4.9. Potassium cyanide (KCN) reagent grade.

4.10. Hydrochloric acid (HCl) concentrated reagent grade.

4.11. Sodium Hydroxide Solution (1.0N)

Dissolve 40.0 g of sodium hydroxide in 800 ml of distilled water, cool and make up to 1 liter in a volumetric flask.

4.12. Sodium Hydroxide Solution (0.01N)

Dilute 10 ml of 1.0N sodium hydroxide solution to 1000 ml with distilled water.

4.13. Chloramine-T solution (0.4%)

Dissolve 2.0 g of chloramine-T in distilled water and dilute to 500 ml.

4.14. Phosphate Buffer, pH 5.2

Dissolve 13.60 g of potassium dihydrogen orthophosphate and 0.28 g of disodium hydrogen phosphate in 900 ml of distilled water and make up to 1 liter in a volumetric flask.

4.15. Buffered Chloramine-T Solution

Add 200 ml of chloramine-T solution to 800 ml of phosphate buffer solution and mix thoroughly.

4.16. Pyridine-Barbituric Acid Reagent

Place 15.0 g of barbituric acid into a 1 liter volumetric flask, add 100 ml of distilled water and mix to thoroughly wet and disperse lumps of the barbituric acid. Add 15 ml of concentrated hydrochloric acid and mix well. Add 75 ml of pyridine and mix. Add 800 ml of distilled water and stir until the barbituric acid has dissolved. Dilute the contents of the flask to 1.0 liter with distilled water. Store in a cool dark place.

NOTE: BARBITURIC ACID IS A RESTRICTED CHEMICAL AND MUST BE STORED IN A LOCKED CABINET.

4.17. Phosphoric Acid, 10%

Dilute 100 ml of phosphoric acid, 85%, to 1 liter with distilled water.

4.18. Cyanide Stock Solution, 100 mg/l as CN

Dissolve 0.250 g of potassium cyanide in 600 ml of distilled water containing 100 ml of 1.0N sodium hydroxide solution. Dilute to 1 liter in a volumetric flask and mix thoroughly.

NOTE: CYANIDE IS EXTREMELY TOXIC AND MUST BE HANDLED WITH CARE TO AVOID CONTACT AND/OR INGESTION OF THE DRY POWDER OR CONCENTRATED SOLUTION.

4.19. Cyanide Intermediate Solution, 10 mg/l as CN

Pipette 100.0 ml of cyanide stock solution into a 1 liter volumetric flask containing 90 ml of 1.0N sodium hydroxide solution, dilute to volume with distilled water and mix thoroughly.

4.20. Working Cyanide Standard Solutions

Dilute 10.0, 20.0, 30.0 and 40.0 ml aliquots of the cyanide intermediate standard and 9 ml of 1.0N sodium hydroxide to 1.0 liter in volumetric flasks, to give working standards of 0.1, 0.2, 0.3 and 0.4 mg/l as CN in 0.01N sodium hydroxide. Standards are stable provided that the bottles are capped when not in use and may be used for several weeks without serious loss of cyanide.

4.21. Quality Control Solutions

Since cyanide is both a perishable and convertible parameter, quality control samples containing other than free cyanide are not appropriate for free cyanide analysis.

Pure standards of at least one month's supply are prepared to give cyanide concentrations of 10% and 80% of the operating range of the automated colorimetric procedure. These solutions are stable for several weeks provided proper preservation procedures are followed.

5. Procedure

- 5.1. Set up the manifold as shown in Figure 3.
- 5.2. Assemble the dialyser blocks.
- 5.3. Ensure that the dialysers are inserted into the flow manifold as shown so that the recipient stream (0.01N sodium hydroxide) flows through the lower channels.
Ensure that the flow of the recipient and sample streams are in opposite directions through the dialyzer blocks.
- 5.4. After a 30 minute warm-up for the colorimeter, run a baseline with all reagents, feeding distilled water through the sample line.
- 5.5. Calibrate the system according to established AutoAnalyzer procedures using the working standards.
- 5.6. Run QC samples to ensure correct response before proceeding with sample analysis.
- 5.7. Run samples in groups of 10, separated by at least one check standard.
- 5.8. Compare the results of unknown samples to the standard curve and report as mg/l free cyanide.

6. Calculation and Reporting

Free cyanide concentrations are calculated as follows:

$$\text{mg/l as CN} = r \times \text{DF}$$

Where:

r = chart reading in mg/l as CN

DF = dilution factor

Results are reported to 2 significant figures.

7. Precision and Accuracy

The automated colorimetric procedure for cyanide has a standard deviation of 0.005 mg/l as CN based upon replicate standards.

Calibration accuracy is controlled by two independently prepared standards at 10% and 80% of the range.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater, 14th edition, APHA, Washington, D.C. 370-372.
- 8.2. Technicon AutoAnalyzer II Methodology, Industrial Method No. 119-71W/Preliminary, Oct. 1971; No. 353-75 W/A, May 1976.
- 8.3. U.S. Environmental Protection Agency (1979). Methods for Chemical Analysis of Water and Wastes. Environmental Monitoring and Support Laboratory, EPA Environmental Research Center, Cincinnati, Ohio. EPA-600/4-99-020.
- 8.4. Hipfner, J.C. Ontario Ministry of the Environment, Laboratory Services Branch, Rexdale, Ontario. Cyanide Speciation using Automated Procedures in Combination with Membrane Diffusion Techniques. Presented at FACSS Conference, Philadelphia, Pa. Sept. Oct. 1980.

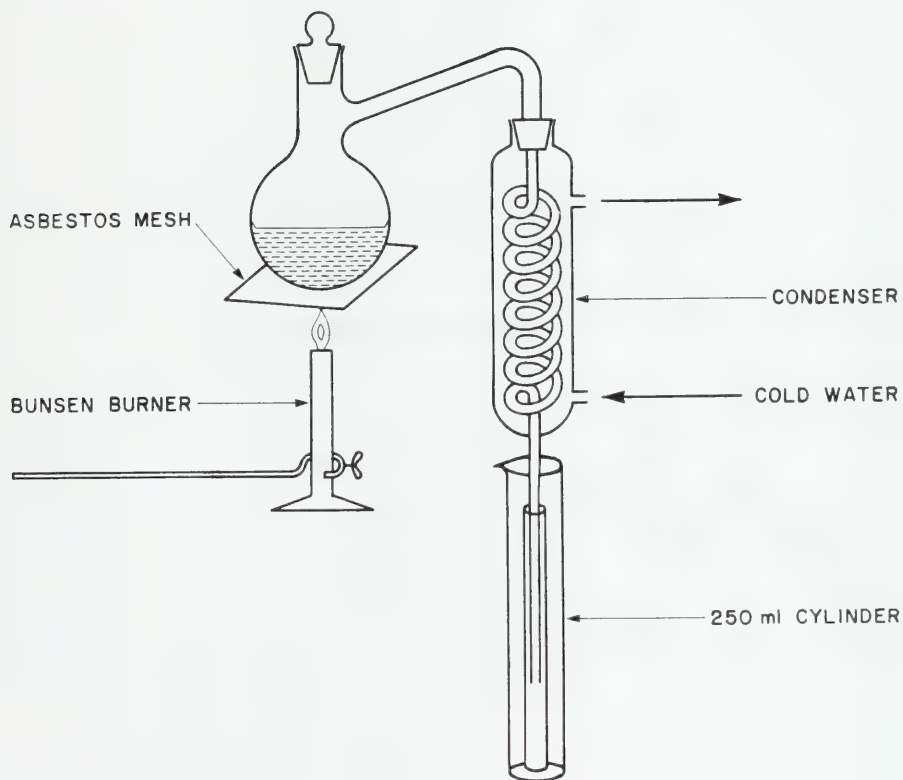


FIGURE 1 — MANUAL DISTILLATION APPARATUS FOR TOTAL CYANIDE

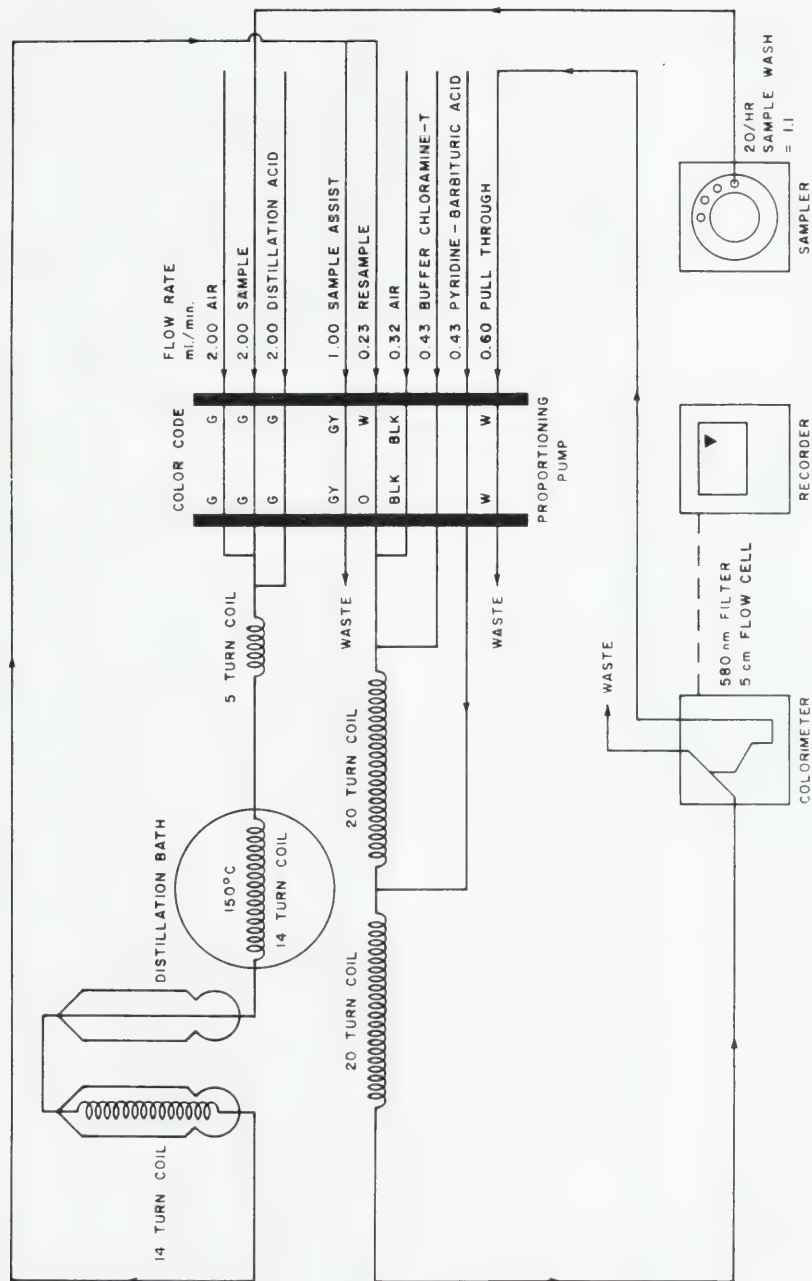


FIGURE 2 — AUTOANALYZER AAT SYSTEM FOR TOTAL CYANIDE DETERMINATION.

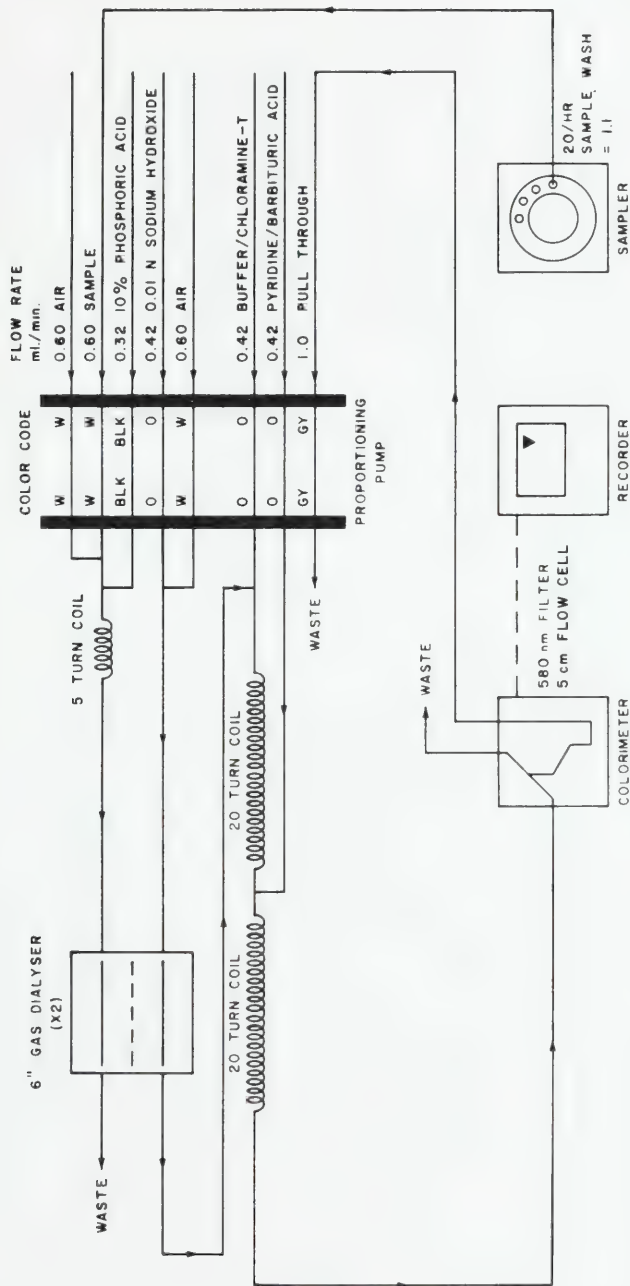


FIGURE 3 — AUTOANALYZER AATII SYSTEM FOR FREE CYANIDE DETERMINATION.

THE DETERMINATION OF DUSTFALL

Air pollutants in the form of dust can be a nuisance factor. Human activity is by far the greatest contributor to total dustfall. Very rarely are significant amounts contributed by wind or volcanic activity.

Dust damages clothing and buildings, contaminates residential areas and causes eye injuries. Dustfall can also damage agricultural and ornamental plants thus lowering their value.

When assessing damages or injuries attributable to dust, an analysis of the elemental composition and a microscopic examination assists in pinpointing the possible toxic agents and their source.

Though the ability of a dust particle to settle is mainly determined by its weight and size (surface area) many other phenomena play a part (wind, precipitation, type of target etc.). It is, therefore, difficult to obtain dustfall data independent of external conditions.

Sample Handling and Preservation

Dustfall

Analyze samples as soon as possible, normally within 1 week of collection. ASTM recommends addition of water to the dustfall jar prior to installation. However, this is not always practical, and is not part of this method.

In cold weather it is suggested that an anti-freeze such as isopropyl alcohol, ethyl alcohol or ethylene glycol, be added to prevent freezing of water in the container. During the summer months algae or fungi grow within the collector. Copper sulphate solution (15 mg/l) or mercuric chloride (0.1 g) may be used as an algicide. The addition of inhibitors, however, presents problems in analytical procedures and contaminates the containers. Inhibitors should be avoided except in extreme cases.

Selection of Method

The "Standard Method for Collection and Analysis of Dustfall" is designated in the ASTM Handbook as D1739-70. The described analytical procedure closely follows the ASTM method, but sampling differs in that water is not added to the dustfall jar prior to exposure, and a polyethylene liner is used.

Laboratory results show that wet and liner type dustfall show good agreement. Day containers give results some 15% below those of wet.

DUSTFALL

Settleable Particulate ASTM Method A

SUMMARY

Matrix	This method is used on settleable air particulate matter.
Substance determined.	The weight of the settleable particulate collected. Chemical analyses for special studies may be performed on the soluble and insoluble fractions.
Interpretation of results.	The weight of the combined soluble and insoluble fractions is converted to $\text{g/m}^2/30$ days and is used to express the atmospheric fall-out of dust within specific areas.
Principle of method.	The sample is filtered to obtain soluble and insoluble fractions. The fractions are dried, weighed, recorded and calculations made.
Time required for analysis.	Samples are run in batches and require approximately 40 minutes per analysis.
Range of application.	The lower limit of measurement is dependent only upon the accuracy of the balance.
Standard deviation.	The analysis cannot be repeated, thus no standard deviations can be given.
Accuracy.	97.7% recovery on simulated dustfall samples.
Detection criteria.	1 mg.
Interferences and shortcomings.	Extraneous interferences could occur and in many cases remain undetected. Materials most likely to contaminate during the period of exposure are insects, tree foliage, bird droppings, algae and fungi.
Minimum volume of sample.	If the collector is dry after the collection period 250 ml of distilled water are added and the jar is set aside for 24 hours.
Preservation and sample container.	Prolonged storage is not recommended because of algal growth and the possible chemical breakdown of organic material.
Safety considerations.	Regular laboratory precautions.

DETERMINATION OF DUSTFALL

ASTM Method A

1. Introduction

Dustfall samples are removed from the liner by treatment in an ultrasonic bath, followed by repeated washing with distilled water and separated by filtration into soluble and insoluble fractions. The weight of the soluble and insoluble fractions is determined separately. The sum of the fractions provides the total weight of the settleable particulate falling on the area being monitored. Current methods do not enable distinction between dustfall and atmospheric pollutants which precipitate with rain and snow and remain after complete evaporation of the volatile parts.

2. Interferences and Shortcomings

While dustfall data is quite variable within zones, and the technique less than precise, dustfall trends can be established over a long period. The method is susceptible to meteorological variations.

3. Apparatus

- 3.1. Plastic container with lid, 15 cm diameter, 30 cm tall.
- 3.2. Hot Plate.
- 3.3. Buchner Funnel.
- 3.4. Circular Whatman #3 Filter Paper, dia. 12-1/2 cm.
- 3.5. Filtration flask, 1 liter.
- 3.6. Vacuum Pump.
- 3.7. Trap Bottle.
- 3.8. Beakers, 250 ml and 50 ml.
- 3.9. Drying Oven.
- 3.10. Kitchen Spatula.
- 3.11. Circular Screen, 20 mesh.
- 3.12. Graduated cylinder, 1 liter.
- 3.13. Ultrasonic Bath.

4. Reagents

None.

5. Procedure

- 5.1. Place a folded filter paper in a 50 ml beaker. Together with a 250 ml beaker, dry to constant weight and record, identifying the beakers. With the vacuum pump on, spread the filter paper in a Buchner funnel, wetting it lightly to form a secure seal.
- 5.2. Immerse the plastic liner in an ultrasonic bath for 4 minutes. Filter the contents through a 20 mesh screen covering the Buchner funnel. This step removes insects and other large particles such as leaves. Discard the contents on the 20 mesh screen.
- 5.3. Spray the wall of the liner with a small amount of water.
- 5.4. Tilt liner at an angle with open end above circular screen. Spray with a forced stream of distilled water to flush all residues onto filter paper. Do not overflow the funnel.
- 5.5. Suck all the liquid through the wet filter paper and break the vacuum. Carefully fold the wet paper twice and use this surface to wipe the adhering materials from around the inside of the Buchner funnel.
- 5.6. Place paper into the numbered 50 ml beaker and dry at 105°C to a constant weight. The difference in the two weighings is recorded as insoluble matter.
- 5.7. Pour the filtrate into a graduated cylinder, record the total volume and mix well. Take a representative aliquot of approximately 200 ml and transfer to the respective 250 ml beaker, recording the aliquot fraction. Evaporate on the hot plate, but not to dryness. Place in a drying oven and complete drying at 105°C to constant weight.
- 5.8. The water soluble and insoluble materials are reported along with the exposure dates, station number and elapsed time.

6. Calculation and Reporting

Calculate the Dustfall in terms of g/m²/30 days as follows:

$$\text{Dustfall} = \frac{w \times 30}{a \times t} \times 10,000$$

Where:

w = total weight of settleable particulate in grams

30 = constant number of days

10,000 = number of cm² in m².

a = open area of sampling container at top in cm² (182.4 cm²).

t = time of exposure, in days.

the equation becomes:

$$\text{Dustfall} = \frac{w \times 300,000}{a \times t}$$

for a 15.2 cm diameter container this becomes

$$\frac{w \times 1644.7}{t}$$

7. Precision and Accuracy

Tests conducted on replicate samples by various air pollution agencies show a precision of 15% for a given type of collection jar and retention fluid.

Tests on the accuracy of the analytical procedure shows the average recovery on simulated dustfall samples to be 97.7%.

8. Bibliography

- 8.1. American Society for Testing Materials (1975). Standard method for collection and analysis of dustfall (settleable particulates). D. 1739-70. ASTM Standards, Part 26, 340.

THE DETERMINATION OF FLUORIDE

Fluoride occurs naturally in ground waters, soils and sediments, primarily as a result of minerals such as fluorspar, fluorapatite, fluorite and cryolite. Most ground waters in Ontario contain fluoride in concentrations of less than 2 mg/l, although higher levels may be found in localized areas containing fluoride bearing rocks.

The fluoridation of municipal water supplies has been widely adopted in Ontario for the prevention of dental caries. The recommended average concentration of fluoride in Ontario drinking water is 1.0 mg/l. Operating limits for municipal supplies are set between 0.8 and 1.2 mg/l. Waters with fluoride levels of greater than 2.4 mg/l are not suitable for use as a municipal water supply. Fluoride concentrations greater than 2 mg/l can produce mottling of teeth, and levels exceeding 50 mg/l may be toxic.

In vegetation, naturally occurring fluoride concentrations do not exceed 10 µg/g (of dry weight) although fluoride concentrations in the surrounding soil may be as high as 500 µg/g. Fluoride damage to vegetation is usually the result of air borne fluoride emissions which may cause acute or chronic damage depending on the length and concentration of fumigation.

The primary aluminum industry is the major source of atmospheric fluoride emissions. However, phosphate fertilizer production, elemental phosphorus plants, brickworks and the primary iron and steel industry are also significant contributors.

Sample Handling and Preservation

Water

Glass or plastic bottles are satisfactory for the fluoride levels routinely encountered in domestic water supplies. If high levels are expected such as from an industrial source, plastic bottles should be used as glass will decompose in the presence of excessive fluoride. Bottles should be rinsed with a portion of sample prior to sample collection. Preservatives are not recommended.

Vegetation

Vegetation samples are collected and transported in perforated plastic bags. No chemical preservatives are required, however, samples should be refrigerated as soon as possible and may be stored in this manner for several weeks. If a longer storage period is necessary, dry samples in a forced draft oven at 80°C for 30 hours, grind in a Wiley mill to pass a 80 mesh screen, homogenize and store in glass jars.

Soils

Soil samples can be collected in polyethylene bags, air dried, ground and stored in glass jars.

Ambient Air

Plastic extensions on the end of each candle facilitate handling, and prevent contamination and loss of coating. Prior to exposure store candle in a sealed, tubular container. After exposure, insert into same container and seal until analyzed.

Selection of Method

Among the many methods suggested for the measurement of fluoride in environmental samples, the colorimetric and the specific ion electrode methods are the most extensively used at present.

Method A is an automated alizarin blue colorimetric method, currently used for the analysis of water samples. Samples undergo a preliminary distillation to remove interferences and fluoride is then determined colorimetrically after the formation of a ternary alizarin blue-lanthanide-fluoride complex.

Method B, the specific ion electrode, is also used for fluoride measurements on water samples. Interferences are eliminated by the addition of a total ionic strength adjustment buffer rather than by distillation.

Method C is used for fluoride determinations on vegetation, soil and sediment samples and involves the same colorimetric procedure as employed in Method A, however extensive sample preparation procedures are required to eliminate interferences and solubilize the fluoride.

Method D provides a measurement of the fluoridation rate of the atmosphere.

FLUORIDE

Automated Alizarin Blue Colorimetric Method A

SUMMARY

Matrix.	This method is used for the measurement of fluoride in domestic water and surface water samples.
Substance determined.	Fluoride ion (F^-), distilled from sulphuric acid at 160 °C.
Interpretation of results.	Results are reported in mg/l F. The recommended fluoridation level in Ontario is 1.0 ± 0.2 mg/l.
Principle of method.	Samples are automatically diluted, digested and distilled from sulphuric acid to remove interferences. The distillate is reacted with color reagent containing lanthanum nitrate and Alizarin Blue to form a ternary Alizarin Blue-lanthanide-fluoride complex which is measured colorimetrically at 630 nm.
Time required for analysis.	Approximately 14 samples can be analyzed in an hour. In one day approximately 200 can be analyzed.
Range of application.	The routine working range is 0.01 - 2.0 mg/l F. This may be extended to a lower range for special samples and to a higher level by dilution.
Standard deviation.	Standard deviations are 0.009 for 0 - 20% of the range; 0.013 for 20 - 50% of the range and 0.018 for 50 - 100% of the range based on within run duplicate samples.
Accuracy.	Accuracy is controlled by 2 long-term quality control standards (QC-A and QC-B) such that $A + B$ and $A - B$ do not vary by more than .08 mg/l (3 standard deviations) from their long-term means.
Detection criterion.	0.015 mg/l.
Interferences and shortcomings.	<p>Interferences from such things as aluminum, iron, lead, zinc, cobalt and copper are removed by the digestion and distillation process. This process is effective even if these metals are present in abundance as in industrial effluents.</p> <p>Extremely acid or basic sample conditions adversely affects the recovery of fluoride during the distillation process. Such samples are adjusted to a neutral pH prior to analysis.</p>

Minimum volume of sample. 50 ml.

Preservation and sample container. Plastic and glass bottles are acceptable for the fluoride levels routinely encountered in domestic water supplies. If high levels are expected, plastic bottles should be used as glass will be attacked by high fluoride concentrations. Bottles previously containing samples with high fluoride levels must not be re-used.

Safety considerations. Safety precautions should be taken when handling the concentrated acids and alkalis used in reagent preparations.

FLUORIDE

Automated Alizarin Blue Colorimetric Method A

1. Introduction

A sample aliquot is processed through an AutoAnalyzer AAI system, where it is distilled from sulphuric acid at a temperature of 160°C and reacted with a color reagent containing Alizarin Blue, lanthanum nitrate, acetone, t-butyl alcohol, ammonium acetate and sodium acetate buffer. The blue color developed is then measured colorimetrically using a 5.0 cm flow cell at a wavelength of 630 nm. Signal strength is displayed on a chart recorder and sample concentrations are measured by comparison of sample peak heights to those of a set of known standards.

2. Interferences and Shortcomings

Sample pH, if significantly different from pH 7 may adversely affect the recovery of fluoride during the distillation process.

Heavy metals interfere with the recovery of fluoride and a distillation step must be employed to remove such metals as aluminum, iron, lead, zinc, cobalt and copper. The distillation process is effective even when these elements are in abundance, as in the case of industrial effluents.

Fluoride bound in an organic matrix is not released by this method, however, this form of fluoride is rarely found in drinking waters.

3. Apparatus

3.1. AutoAnalyzer AAI system composed of the following modules:

- 3.1.1. sampler
- 3.1.2. proportioning pump
- 3.1.3. heating bath, high temperature, with thermostatic temperature control and micro distillation head.
- 3.1.4. colorimeter, with 630 nm filters and 5.0 cm flow cell.
- 3.1.5. voltage regulator
- 3.1.6. chart recorder

3.2. Pump tubing and associated manifold glassware as in Figure F 1.

3.3. Test tubes, 100 x 22 mm.

3.4. Culture tube racks.

3.5. Reagent bottles, polyethylene, 1 liter capacity.

3.6. Reagent reservoir bottles, 1 liter, 2 liter.

3.7. Volumetric flasks, 250 ml, 500 ml, 1 liter.

4. Reagents

4.1. Lanthanum nitrate ($\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$), reagent grade crystals.

4.2. Sodium acetate trihydrate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$), reagent grade crystals.

4.3. Sodium fluoride (NaF), anhydrous, reagent grade crystals.

4.4. 3-aminomethylalizarin-N,N-diacetic acid, reagent grade crystals. (Also known as Alizarin Blue, Alizarin Fluorine Blue and Alizarin Complexone.)

4.5. Acetic acid (CH_3COOH), reagent grade, glacial.

4.6. Sulphuric acid (H_2SO_4), concentrated reagent grade.

4.7. Ammonium hydroxide (NH_4OH), concentrated reagent grade.

4.8. Acetone (CH_3COCH_3), reagent grade.

4.9. 2-methylpropan-2-ol ($(\text{CH}_3)_3\text{COH}$), (tertiary butyl alcohol), reagent grade.

4.10. Fluoride Stock Solution (100 mg/l F)

Dissolve 0.22101 ± 0.00005 g anhydrous sodium fluoride, dried at 150°C for 1 hour and cooled in a desiccator, in distilled water and dilute to 1 liter.

4.11. Intermediate Fluoride Solution (20 mg/l F)

Dilute 200 ml fluoride stock solution to 1 liter with distilled water.

4.12. Fluoride Working Standard Solutions

Dilute aliquots of 5.0, 10.0, 20.0, 40.0, 60.0, 80.0 and 100.0 ml intermediate fluoride solution to 1000 ml. This gives a series of working solutions with fluoride concentration of 0.1, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 mg/l respectively. Store solutions in plastic bottles.

4.13. Quality Control Stock Solution

Dry anhydrous sodium fluoride at 150°C for 1 hour and cooled in a desiccator. Weigh out 0.13261 ± 0.00005 g dried sodium fluoride, dissolve in distilled water and dilute to 1 liter.

4.14. Quality Control Working Solutions.

QC-A: Dilute 25 ml quality control stock solution to 1 liter with distilled water. This gives a fluoride concentration of 1.5 mg/l.

QC-B: Dilute 5 ml quality control stock solution to 1 liter with distilled water. This gives a fluoride concentration of 0.30 mg/l.

Discard remaining Quality Control stock solution.

4.15. Sensitivity Standards

High: Dilute 200 ml intermediate fluoride stock solution to 2 liters with distilled water to give a fluoride concentration of 2.0 mg/l.

Low: Dilute 20 ml intermediate fluoride stock solution to 2 liters with distilled water to give a fluoride concentration of 0.2 mg/l.

4.16. Distillation Acid

Slowly add 30 ml concentrated sulphuric acid to approximately 500 ml distilled water. Mix thoroughly, add 6 ml fluoride stock solution and dilute to 1 liter. This reagent has a fluoride concentration of 0.6 mg/l.

4.17. Buffer Stock Solution

Dissolve 240 g sodium acetate trihydrate and 400 ml glacial acetic acid in distilled water and dilute to 4 liters.

4.18. Lanthanum Stock Solution

Dissolve 2.16 g lanthanum nitrate in distilled water and dilute to 500 ml. Refrigerate this solution when not in use.

4.19. Stock Color Reagent

Add 2.130 g Alizarin Blue and 4.4 ml concentrated ammonium hydroxide to 100 ml distilled water. Mix well until dye is completely dissolved. To this mixture add 4.4 ml glacial acetic acid and dilute to 500 ml with distilled water. Refrigerate this solution when not in use.

4.20. Working Color Reagent

Combine 300 ml buffer stock solution, 150 ml acetone, 50 ml 2-methylpropan-2-ol (tertiary butyl alcohol), 40 ml stock color reagent and 40 ml lanthanum stock solution. Dilute to 1 liter with distilled water. Refrigerate this solution when not in use.

NOTE: Tertiary butyl alcohol is solid at room temperature and must be warmed slightly before use.

5. Procedure

REFER TO MANUFACTURER'S MANUAL FOR CLEANING, SET-UP AND CHECKING PROCEDURES FOR AUTOANALYZER SYSTEM.

- 5.1. Rinse 100 x 22 mm test tubes with approximately 10 to 15 ml well shaken sample. Discard this portion of sample.
- 5.2. Fill rinsed test tube with sample and place in sampler.
- 5.3. Each run of samples should include the following:

Set of standards (STDs); distilled water blank (Bl); quality control samples (A and B); sensitivity monitoring standards (H and L); samples in groups of 10 or less.

Load samples in the following sequence: 2H; Bl; STDs; Bl; A; B; Bl; n((10 x sample, Bl); (10 x sample, L, H, Bl)). Where n = number of repetitive sets of samples.
- 5.5. Set AutoAnalyzer into operation. Prepare a calibration curve for the series of standards. Ensure that blanks and quality control checks agree with previous results.
- 5.6. Record measured values of QC-A and QC-B and the STD CAL setting during the run. Record values of the Highs and Lows and determine whether a sensitivity correction should be applied.

5.7. Read sample peak from calibration chart and record the result.

6. Calculation and Reporting

The reading recorded in 5.7 is multiplied by the dilution factor DF:

$$DF = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

Results are reported to 3 significant figures over the full concentration range. Samples with a fluoride concentration less than the detection criterion have the remark <T appended to the result.

7. Precision and Accuracy

Standard deviations based on within-run duplicates in the 0.01 to 2.00 mg/l range are: 0.009 for 0 - 20% of the range; 0.013 for 20 - 50% of the range and 0.018 for 50 - 100% of the range.

Accuracy is controlled by 2 independently prepared long-term standards (QC-A and QC-B) with concentrations of 1.5 mg/l and 0.030 mg/l fluoride. Calibration is controlled in such a way that (A + B) and (A - B) do not vary by more than .08 mg/l (3 standard deviations) from their long-term means.

8. Bibliography

- 8.1 American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Waters and Wastewaters. 13th edition, APHA, Washington, D.C.
- 8.2 Chan, K.M. and Riley, J.P. (1966). The automatic determination of fluoride in seawater and other natural waters. *Analytica Chimica Acta* 35: 365.
- 8.3 Mandl, R.H., Weinstein, L.H., Jacobson, J.S., McCunan, D.C. and Hitchcock, A.E. (1966). Simplified semi-automated analysis of fluoride. Automation in Analytical Chemistry, Mediad Inc. 270.
- 8.4 Technicon Industrial Systems (1972). Fluoride in Water and Wastewater, Industrial Method No. 129-71W. Technicon Industrial Systems, Tarrytown, New York.
- 8.5 United States Department of Commerce (1967). Trace Characterization, Chemical and Physical. National Standards Monograph 100, United States Department of Commerce. 225.

FLUORIDE

Specific Ion Electrode Method B

SUMMARY

Matrix.	This method is routinely used for fluoride measurements on domestic water in the London and Kingston Regional Laboratories.
Substance determined.	Fluoride ion (F^-).
Interpretation of results.	The fluoride ion electrode measures the total fluoride in a sample, since any complexed fluoride is dissociated by using a total ionic strength adjustment buffer.
Principle of method.	The method is based on the measurement, in millivolts, of the activity of the fluoride ion in the solution. The concentration of fluoride is a logarithmic function of the millivolt reading. (That is, 10 fold changes in concentration represent equal changes in millivolt readings). The meter used, however, converts the millivolt readings and reports results in mg/l F.
Time required for analysis.	Approximately 5 minutes are required for a single analysis. About 100 analyses can be performed in a day.
Range of application.	0.1 - 10,000 mg/l for a 25 ml aliquot. The majority of samples analyzed, however, fall within 0.1 mg/l and 2 mg/l F.
Standard deviation.	For the 0.1 - 2.0 mg/l range standard deviations of within run duplicates are: 0.003 for 0 - 20% of range; 0.003 for 20 - 50% of range and 0.0012 for 50 - 100% of range.
Accuracy.	Accuracy is controlled by 2 independently prepared long-term standards (QC-A and QC-B) such that $(A + B)$ and $(A - B)$ are within 0.041 mg/l (3 standard deviations) of their long-term means.
Detection criteria.	The detection limit is 0.1 mg/l F. Although 0.01 mg/l can be read, results of less than 0.1 mg/l are not reported due to a slight curvature at the bottom of the calibration curve.
Interferences and shortcomings.	Hydroxide ion and complexing metal interferences are removed by the addition of total ionic strength adjustment buffer to all samples and standards.
Minimum volume of sample.	For most samples 50 ml is required.

**Preservation and
sample container.**

Polyethylene and glass bottles are acceptable if precautions are taken to prevent the use of containers which previously contained high fluoride solutions.

**Safety
considerations.**

Normal laboratory safety precautions should be observed. Care should be taken when dealing with strong alkalis and concentrated acids.

FLUORIDE

Specific Ion Electrode Method B

1. Introduction

An aliquot of sample (25 ml) is diluted with an equal portion of total ionic strength adjustment buffer (TISAB). The fluoride ion electrode and reference calomel electrode are immersed in the test sample and continuous stirring is applied. The resulting potential established by the free or unbound fluoride ions is converted and displayed as mg/l fluoride on an Orion 901 specific ion meter.

2. Interferences and Shortcomings

Although most of the interferences are removed by the addition of an equal volume of total ionic strength adjustment buffer (TISAB) solution, a 5% depression in the electrode response has been observed on an undistilled 1.0 mg/l fluoride standard in the presence of 1.0 mg/l aluminum. In most samples, however, aluminum is not present in high enough concentrations to cause problems.

3. Apparatus

- 3.1. Beakers, glass or plastic, 100 ml.
- 3.2. Magnetic stirrer.
- 3.3. Fluoride specific ion electrode.
- 3.4. Reference electrode.
- 3.5. Specific ion meter, digital, Orion Model 901.

4. Reagents

- 4.1. Sodium fluoride (NaF), anhydrous, reagent grade crystals.
- 4.2. Sodium hydroxide (NaOH), reagent grade pellets.
- 4.3. Sodium chloride (NaCl), reagent grade crystals.
- 4.4. 1,2-cyclohexylenedinitrilo tetraacetic acid (CDTA), reagent grade crystals.
- 4.5. Acetic acid (CH_3COOH), glacial, reagent grade.
- 4.6. **Total Ionic Strength Adjustment Buffer (TISAB)**

Place approximately 1 liter distilled water in a 3 liter container. While stirring, add 171 ml glacial acetic acid, 174 g sodium chloride and 12 g cyclohexylenedinitrilo tetraacetic acid (CDTA). Slowly add 95 g sodium hydroxide pellets and allow the solution to cool. Adjust the pH to between 5.0

and 5.5 using sodium hydroxide or hydrochloric acid as required. (The pH is monitored by immersing pH electrodes directly into the solution.) Dilute to 3 liters with distilled water.

4.7. Fluoride Stock Solution (100 mg/l F)

Dissolve 0.2210 g anhydrous sodium fluoride in distilled water and dilute to 1 liter with distilled water. Prepare a similar quality control stock solution using a different batch of sodium fluoride.

4.8. Fluoride Standard Solutions

To 10 and 100 ml fluoride stock solutions add 25 ml TISAB and dilute to 1 liter with distilled water to give a series of standards with concentrations of 1 and 10 mg/l fluoride respectively.

4.9. Fluoride Quality Control Solutions

Using a new batch of sodium fluoride, prepare a QC-A solution with a fluoride concentration of 1.4 mg/l and a QC-B solution of 0.6 mg/l fluoride.

5. Procedure

5.1. Transfer 25 ml of sample, standards and quality control solutions to 100 ml beakers and add 25 ml TISAB solution to each beaker.

5.2. Place electrode in the 1.0 mg/l fluoride standard, stir and using the CALIBRATION setting adjust meter to read 1.0 mg/l.

5.3. Place electrode in the 10.0 mg/l fluoride standard, stir and, using the SLOPE CONTROL adjust meter to read 10 mg/l.

NOTE: Calibration of the meter in this way eliminates the need to accommodate for the dilution factor produced by the addition of the TISAB solution.

5.4. Read and record the fluoride concentration of quality control solutions A and B.

5.5. Immerse electrodes into sample solution to be measured, stir, read and record fluoride concentration. Rinse and blot dry electrodes between measurements.

NOTE: When measuring a sample having a low fluoride concentration after a very high sample, allow a few minutes for the system to stabilize.

6. Calculation and Reporting

Report results as follows:

Result:

0.1 mg/l F
0.1 - 9.9 mg/l F
9.9 mg/l F

Report:

as 0.1 mg/l
to nearest 0.1
to 2 significant figures

7. Precision and Accuracy

For the 0.1 - 2.0 mg/l fluoride range, standard deviations of within run duplicates are: 0.003 for 0 - 20% of range; 0.003 for 20 - 50% of range; and 0.0012 for 50 - 100% of range.

Calibration is controlled by 2 independently prepared long-term standards (QC-A and QC-B) with fluoride concentrations of 1.4 and 0.6 mg/l, such that (A + B) and (A - B) are within 0.041 mg/l (3 standard deviations) of their long-term means.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Waters and Wastewaters. 13th edition, APHA, Washington, D.C.
- 8.2. Orion Research (1973). Analytical Methods Guide. 6th edition, Orion Research.

FLUORIDE

Alkali Fusion-Automated Alizarin Blue Colorimetric Method C

SUMMARY

Matrix.	This method is used for fluoride determinations on vegetation, soil and sediment samples.
Substance determined.	Fluoride ion (F^-), as distilled from 10% v/v sulphuric acid digestion at 170°C.
Interpretation of results.	Results are reported as $\mu\text{g/g F}$. Fluoride fumigations may cause aesthetic damage resulting from long-term fluoridation, and accumulation of fluoride in forage grass which may be injurious to grazing cattle.
Principle of method.	Dried, homogenized vegetation samples or air dried ground soil samples are treated with an excess of calcium hydroxide to bind volatile fluoride during ashing. Samples are dried and ashed in a muffle furnace at 525°C to convert all fluoride into an inorganic form. Ashed samples are fused with sodium hydroxide to convert fluoride into a soluble form. Fused samples are dissolved (or suspended) in aqueous solution and analyzed in an automated system involving sample distillation to remove interfering elements and reaction of the distillate with lanthanum nitrate and alizarin blue. The intensity of the blue complex developed is measured spectrophotometrically at 620 nm and the results compared with the response of undigested, aqueous fluoride standards.
Time required for analysis.	A single operator can process up to 250 samples per week.
Range of application.	Based on 1 g dried vegetation and 0.2 g dried soil or sediment converted to 50 ml of aqueous suspension the routine working range is 3 - 200 $\mu\text{g/g}$ fluoride. Samples in excess can be brought to within this range by dilution.
Standard deviation.	Based on 577 pairs of vegetation duplicates in the 3 to 150 $\mu\text{g/g}$ fluoride range, the relative standard deviation is 9.5%.
Accuracy.	Calibration is maintained by 2 long-term standards QC-A and QC-B such that the measured value of these standards is within 2 standard deviations for the long-term mean value of these standards.
Detection limit.	The detection limit is 3 $\mu\text{g/g}$ dry weight.

Interferences and shortcomings.	Chloride if present at concentrations of 5% of dried weight, causes minor negative interference. Sample pretreatment eliminates heavy metal and organic matter interference.
Minimum volume of sample.	At low fluoride levels, a minimum of 1 g of dried vegetation and 0.2 g soil or sediment is required. For a representative sample, a homogenized dried vegetation sample of at least 20 g (approximately 200 g fresh material) is required and about 10 g soil or sediment.
Preservation and sample container.	Collect vegetation samples in perforated, polyethylene bags and refrigerate. Oven dry, grind to less than 80 mesh and store in glass jars under dry conditions. Collect soil samples in pomade jars. Air dry and grind to less than 2 mm.
Safety considerations.	Splash protective eye shields should be used when using strong acids and bases. A full face shield should be worn for sodium hydroxide fusion.

FLUORIDE

Alkali Fusion-Automated Alizarin Blue Colorimetric Method C

1. Introduction

A 1 g sample of dried, ground and homogenized vegetation or 0.2 g of air dried and ground soil is treated with 10 ml saturated calcium hydroxide solution. The sample is oven dried at 105°C, transferred to a muffle furnace and ignited at 525°C for 2 hours. Sodium hydroxide pellets are then added and the ashed sample is fused in the muffle furnace for 3 minutes. The sample is cooled, 25 ml of distilled water is added, the sample is left to dissolve for 3 hours and is then transferred to a 50 ml volumetric flask.

The digested sample is transferred to an AutoAnalyzer system where it is mixed with 10% sulphuric acid and distilled at 170°C. The distillate is reacted with a lanthanum nitrate complex of alizarin blue in a mixed acetone-butanol-water system, buffered with acetic acid and sodium acetate. The absorbance of the fluoride blue complex is measured spectrophotometrically at 620 nm.

2. Interferences and Shortcomings

Interferences due to variations in pH and organic bound fluorine are eliminated due to the rigorous digestion procedure used. Chloride, if present at 5% of the dried weight level, slightly depresses the response of the method to fluoride. However, when chloride is present at high levels, oxidizing elements such as manganese have been observed to generate interferences in the form of negative peaks.

3. Apparatus

3.1. AutoAnalyzer II system comprised of the following modules:

- 3.1.1 sampler
- 3.1.2 proportioning pump
- 3.1.3 heating bath, heating to 170°C equipped with thermostatic control and microdistillation head.
- 3.1.4 colorimeter fitted with 620 nm filters and a 1.5 cm flow cell
- 3.1.5 voltage regulator
- 3.1.6 chart recorder

3.2. Pump tubing and assorted manifold glassware as in Figure F 2.

3.3. Muffle furnace and controller with temperature range to 1000°C.

3.4. Drying oven, heating to 105°C.

3.5. Balance, top loading, accurate to ± 1 mg with tare.

3.6. Nickel crucibles, 50 ml, low form.

- 3.7. Flasks, volumetric, plastic, assorted sizes.
- 3.8. Pipettes, volumetric, assorted sizes.
- 3.9. Test tubes, 85 x 14 mm.
- 3.10. Test tube rack, 40 tube capacity.
- 3.11. Reagent bottles, polyethylene.

4. Reagents

- 4.1. Sodium hydroxide (NaOH), anhydrous, reagent grade pellets.
- 4.2. Sodium fluoride (NaF), anhydrous, reagent grade crystals.
- 4.3. Lanthanum nitrate ($\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$), reagent grade crystals.
- 4.4. Sodium acetate (CH_3COONa), anhydrous reagent grade crystals.
- 4.5. Calcium oxide (CaO) reagent grade powder.
- 4.6. 3-aminomethylalizarin-N,N-diacetic acid, reagent grade crystals. Also known as Alizarin Fluorine Blue.
- 4.7. 2-methylpropan-2-ol ($(\text{CH}_3)_3\text{COH}$), tertiary butyl alcohol, reagent grade.
- 4.8. Acetic acid (CH_3COOH), glacial, reagent grade.
- 4.9. Sulphuric acid (H_2SO_4), concentrated reagent grade.
- 4.10. Ammonium hydroxide (NH_4OH), concentrated reagent grade.
- 4.11. Acetone (CH_3COCH_3), reagent grade.
- 4.12. Brij-35, wetting agent, 30%.
- 4.13. **Fluoride Stock Solution (100 mg/l F)**

Dissolve 0.2210 g anhydrous sodium fluoride in distilled water and dilute to 1 liter.

4.14. Fluoride Working Standard Solutions

Dilute 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 ml aliquots of fluoride stock solution to 500 ml with distilled water. This gives a set of working standards with fluoride concentration of 0.2, 0.4, 1.0, 2.0, 3.0 and 4.0 mg/l respectively. Store working standards in plastic bottles.

4.15. Quality Control Procedure

Quality control solutions A and B are prepared by combining sets of residues from analytical determinations in 500 ml polyethylene bottles until at least one month's supply of solution has been accumulated. Judicious blending of samples will provide controls in the 10% to 20% and 80% to 90% range of the instrumental response without further dilutions.

These quality control solutions are run daily with the calibration standards and serve to detect anomalies in the calibration blanks, systematic errors and as a monitor of the sensitivity response of the instrumentation.

When new A and B controls are required, they should be prepared sufficiently in advance that an overlap of at least three days of comparative data can be acquired. With vegetation samples, A and B solutions are quite stable for at least sixty days.

4.16. Distillation Acid (10% H₂SO₄, 0.5 mg/l F)

Slowly add 100 ml concentrated sulphuric acid to 1000 ml distilled water. Mix thoroughly, cool to room temperature and add 5 ml stock fluoride solution. Mix well.

4.17. Buffer Stock Solution

Dissolve 60 g anhydrous sodium acetate and 100 ml glacial acetic acid in distilled water and dilute to 1 liter.

4.18. Lanthanum Stock Solution

Dissolve 1.08 g lanthanum nitrate in distilled water and dilute to 250 ml. Refrigerate solution when not in use.

4.19. Stock Color Reagent

Suspend 0.96 g alizarin fluorine blue in 100 ml distilled water. Add 2 ml concentrated ammonium hydroxide and shake until dye has completely dissolved. Add 2 ml glacial acetic acid and dilute to 250 ml with distilled water. Store in an amber bottle at 4 °C.

4.20. Working Color Reagent

Mix in the following order: 150 ml buffer stock solution, 75 ml acetone, 25 ml tertiary butyl alcohol, 18 ml stock color reagent, 20 ml lanthanum stock solution and 1 ml Brij-35 (a wetting agent). Dilute mixture to 500 ml with distilled water. Prepare reagent 1 day prior to use and store under refrigeration. If kept refrigerated this reagent is stable for 2 weeks.

NOTE: Tertiary butyl alcohol is a solid at room temperature and must be warmed slightly before use.

4.21. Calcium Oxide Solution (saturated)

Dissolve 20 g calcium oxide in distilled water and dilute to 1 liter. Shake well and allow to stand overnight. Use the supernatant liquid.

5. Procedure

5.1. Sample Preparation

If a washed vegetation sample is being analysed, place a portion of the sample in a 12 inch porcelain dish and gently rinse for 30 seconds with a solution 0.05% Alconox, and 0.05% EDTA tetra sodium salt. Rinse 3 times with 1 liter distilled water and transfer wet material to a 1 liter beaker. If an unwashed sample is being analysed, transfer a portion of sample to a new Kraft paper bag. Dry both washed and unwashed portions in a forced air oven at 80 °C, grind for

48 hours in a Wiley mill to pass through an 80 mesh screen and store in 4 oz screw cap jars.

Soil samples should be air dried and ground to pass through a 2 mm sieve.

5.2. Sample Digestion

5.2.1. Weigh 1.00 g dried vegetation sample or 0.2 g soil sample into a nickel crucible. (Record sample number and corresponding crucible identification number.)

5.2.2. Add 10 ml saturated calcium oxide solution to each sample and allow to stand until the sample is thoroughly wetted. For each batch, prepare 2 blank crucibles.

5.2.3. Transfer samples to a drying oven and dry at 105 °C until completely dry or for at least 2 hours.

5.2.4. Transfer dried samples to a muffle furnace at room temperature and heat to 250 °C. After 15 minutes, raise temperature to 525 °C and maintain this temperature for a further 2 hours. Keep furnace door closed throughout.

5.2.5. Carefully remove crucibles from hot furnace, add 3 g sodium hydroxide pellets and return to oven with door closed for 3 minutes. Remove from oven and swirl until fused melt is partially solidified. Cool crucibles to room temperature and add 25 ml distilled water. Let stand for at least 3 hours until melt has dissolved.

NOTE: Wear face mask throughout this operation due to caustic nature of sodium hydroxide.

5.2.6. By repeated rinsings with distilled water transfer crucible contents to a corresponding clean, numbered 50 ml plastic volumetric flask. Adjust to a final volume of 50 ml with distilled water.

NOTE: Wear eye protection.

5.3. Fluoride Determination

REFER TO MANUFACTURER'S MANUAL FOR SET-UP, CLEANING AND CHECKING PROCEDURES FOR AUTOANALYZER SYSTEM.

5.3.1. Set up AutoAnalyzer system and ensure that heating bath temperature is 170 °C and is stable before proceeding with analysis.

5.3.2. During the daily analysis, run standards frequently to detect changes in sensitivity.

5.3.3. Run samples, blanks and standards in the following sequence:
STD(1).....STD(5); Bl; QC-A; QC-B; Bl; Digested Bl1; Digested Bl2; 10 samples, Bl; 10 samples; Bl; STD(2); STD(4).

5.3.4. Ensure that QC-A and QC-B are within defined acceptable limits prior to continuing analysis.

6. Calculation and Reporting

Peak heights of the standards are used to prepare a calibration curve. For each sample $\mu\text{g/ml F}$ is determined using the calibration curve.

To determine fluoride concentrations in $\mu\text{g/g}$ of sample use the following equation:

$$\mu\text{g/g F} = \frac{(a - b) \times 50 \times c}{d}$$

Where:

a = $\mu\text{g/ml F}$ in sample solution

b = blank reading in $\mu\text{g/ml F}$

c = dilution factor (where applicable)

d = sample weight (g)

Results are reported to the nearest $\mu\text{g/g}$ dry weight of sample.

7. Precision and Accuracy

Based on 577 pairs of vegetation duplicates in the 3 - 150 $\mu\text{g/g}$ fluoride range, the relative standard deviation is 9.5%.

Accuracy is controlled by 2 long-term standards, QC-A and QC-B which are prepared by combining at least 1 month's supply of sample solution to produce at QC-A at approximately 70% and a QC-B at approximately 20% of scale. The quality control solution measurements must not vary by more than 2 standard deviations from their respective long-term means.

8. Bibliography

- 8.1. Chapman, H.D. (1965). Diagnostic Criteria for Plants and Soils. Department of Soils and Plant Nutrition, University of California, Los Angeles, Calif.
- 8.2. National Academy of Science. (1971). Fluorides, Washington, D.C.
- 8.3. Technicon Industrial Systems (19?) Fluoride in Plant Tissue. Method No. 206-72A. Technicon Industrial Systems, Tarrytown, New York.

FLUORIDATION RATE OF THE ATMOSPHERE

Candle - Specific Ion Electrode Method D

SUMMARY

Matrix.	This method is used to determine the fluoridation rate of the atmosphere.
Substance determined.	Fluoride ion, F^- .
Interpretation of results.	Results are reported as $\mu g F^-/100 cm^2/30$ days. Since the candle exposure period is approximately 30 days, it is not possible to determine periods or specific days of high or low fluoride air pollution. The results must be considered as a relative measure of the particular area surveyed.
Principle of method.	Chromatographic filter paper is coated by passing prepared strips through a calcium oxide slurry. This paper is cut to size, wrapped around and attached to a plastic former. On location, it is placed in a louvered shelter for approximately 30 days, after which time, the candle is sealed and returned to the laboratory for fluoride analysis. Fluoride is extracted in a buffer solution and determined by a fluoride specific ion electrode.
Time required for analysis.	One week would be required to prepare, analyze and field handle a particular survey. This is in addition to 30 day exposure period. Work performance has been set at 27 candles per man day on a routine basis.
Range of application.	0.05 mg/l - 5.0 mg/l fluoride in test solution. 2 - 1000 $\mu g F/100 cm^2/30$ days.
Standard deviation.	.034 at the 0.5 mg/l level.
Accuracy.	Not yet available.
Detection criteria.	2 $\mu g/100 cm^2/30$ days.
Interferences and shortcomings.	Impacted fluoride particulate causes an increase in fluoride levels attributed to gaseous pollutants. Static monitors are susceptible to wind velocity and direction variations, surrounding buildings, elevation and humidity. Consequently, 2 candles exposed side by side could vary by as much as 30%.

**Minimum volume
of sample.**

The entire candle coated paper is required for the analysis. The take-up volume is 50 ml.

**Preservation and
sample container.**

The candles are picked up on location and inserted into plastic tubes. The tubes are sealed and shipped or stored.

**Safety
considerations.**

Care should be exercised when handling lime (CaO).

FLUORIDATION RATE OF THE ATMOSPHERE

Candle - Specific Ion Electrode Method D

1. Introduction

The fluoride candle provides a simplified means for measuring gaseous fluoride in ambient air at a specific location. It is indicative of the relative degree of fluoride pollution. The fluoride is collected by exposing a filter paper, presoaked with saturated calcium oxide solution, in the area under survey for approximately 30 days. Fluoride is extracted from the exposed filter paper in a buffer solution and is determined by using a fluoride specific ion electrode in conjunction with a standard, single junction, sleeve-type reference electrode. The potential developed by the presence of fluoride ion is measured by an expanded scale pH/mV meter or by a specific ion meter having a direct concentration scale for fluoride.

2. Interferences and Shortcomings

Polyvalent cations such as Si^{+4} , Fe^{+3} and Al^{+3} interfere by forming complexes with fluoride. The addition of a buffer eliminates these interferences.

3. Apparatus

- 3.1. Plexiglass tube, 31.8 mm O.D. x 3.2 mm wall x 90 mm long.
- 3.2. Plexiglass tube, 22.3 mm O.D. x 1.6 mm wall x 140 mm long.
- 3.3. Adhesive tape, double coated.
- 3.4. Chromatographic sheets, Whatman #4, 46 x 57 cm.
- 3.5. Shipping tubes, polycarbonate, 38 mm O.D. x 1.6 mm wall x 163 mm long.
- 3.6. Caplugs, plastic, #12x and #16.
- 3.7. End caps, plastic, #17-S.
- 3.8. Scissors.
- 3.9. Dispenser, 50 ml (Oxford or equivalent).
- 3.10. Beakers, 50 ml, polyethylene or neoprene.
- 3.11. Water bath.
- 3.12. Squeeze wash bottle.
- 3.13. pH/mV meter or a specific ion meter (Orion microprocessor ionalyzer 901, or equivalent).

- 3.14. Fluoride electrode (Orion 94-09, or equivalent).
- 3.15. Single junction reference electrode (Orion 90-01, or equivalent).
- 3.16. Magnetic stirrer and Teflon coated stirring bar.

4. Reagents

- 4.1. Sodium fluoride (NaF), anhydrous, reagent grade crystal.
- 4.2. Calcium oxide (CaO), (low in fluoride), reagent grade.
- 4.3. Phosphoric acid (H_3PO_4), concentrated, reagent grade (85%).
- 4.4. Sodium hydroxide (NaOH), reagent grade pellets.
- 4.5. Sodium citrate ($\text{Na}_2\text{HC}_6\text{H}_5\text{O}_7 \cdot 1\frac{1}{2}\text{H}_2\text{O}$), reagent grade.
- 4.6. Ethylenediaminetetra-acetic acid (EDTA) disodium salt, reagent grade.
- 4.7. Calcium Oxide Solution (saturated).

4.8. Buffer Solution

Add 3.5 g sodium citrate, 20.00 g sodium chloride, 10.0 g EDTA (disodium salt), 41.7 g sodium hydroxide, and 44.0 ml phosphoric acid (85%) to approximately 850 ml of distilled water. Stir until dissolved and cool to room temperature. Adjust the pH of the solution to 6.7 ± 0.1 with 10M sodium hydroxide solution. Transfer the solution to a 1000 ml volumetric flask and dilute to volume with distilled water.

4.9. Stock Fluoride Solution (1000 mg/l)

Dissolve 2.210 g anhydrous sodium fluoride in distilled water and dilute to 1 liter.

4.10. Standard Fluoride Solution (100 mg/l)

Dilute 100 ml fluoride stock solution to 1 liter with distilled water.

5. Procedure

5.1. Candle Preparation

- 5.1.1. Cut a #4 Whatman chromatographic filter sheet into 11 cm wide strips.
- 5.1.2. Pour saturated calcium oxide solution into a tray, preheat to $40^\circ - 45^\circ\text{C}$. Stir magnetically.
- 5.1.3. Immerse strips into calcium oxide solution for 60 seconds.
- 5.1.4. Use glass rod to smooth the coating to prevent slurry blotching.

- 5.1.5. Hang lime coated strips to dry in a closed room.
- 5.1.6. After drying, cut strips into 9 x 11 cm rectangles and store in an air-tight container.
- 5.1.7. Cut double sided adhesive tape into 5 mm x 9 cm strips.
- 5.1.8. Affix strip of tape to one end of the coated filter paper. Tightly wrap the coated filter paper around the larger diameter plexiglass tube. Draw end without adhesive tape firmly and evenly over the exposed adhesive tape of the bottom 9 cm end to overlap. Press down for good adhesion.
- 5.1.9. Assemble short length, larger diameter plexiglass tubing onto the narrow plexiglass tubing.
- 5.1.10. Insert candle into polycarbonate shipping container and cap at both ends. Each container has an identifying station number on the outside.

5.2. Calibration

- 5.2.1. Cut three 9 x 11 cm strips of unexposed lime paper into small pieces.
- 5.2.2. Place the cut paper into three 100 ml polyethylene or neoprene beakers.
- 5.2.3. Spike 2 beakers with appropriate volumes of standard fluoride solution and bring to 0.2 and 2.0 mg/l F by diluting to 50 ml with buffer solution.

NOTE: For candles containing high fluoride concentrations, standards may be expanded up to 50 mg/l if necessary.

5.3. Fluoride Determination

REFER TO MANUFACTURER'S MANUAL FOR OPERATION AND CALIBRATION OF SPECIFIC ION METER.

- 5.3.1. Carefully remove exposed candle from plastic container.
- 5.3.2. Remove coated paper from candle taking care to avoid any loss of coating. Fold paper with exposed surface inside.
- 5.3.3. Cut paper into 10 mm squares (approximately) over a clean 100 ml neoprene beaker, allowing the cut pieces to fall into the beaker.
- 5.3.4. Using a pipette, add 50 ml buffer solution to beaker.
- 5.3.5. Place beaker into a temperature controlled water bath set at 40°C for 1 hour. Remove beaker and allow solution to cool.
- 5.3.6. Place beaker on a magnetic stirrer and stir at high speed.
- 5.3.7. Immerse electrodes in solution and observe meter reading while stirring.

- 5.3.8. Using CONCENTRATION mode of specific ion meter, record fluoride concentration in unknown sample in mg/l after 3 minutes or after reading has stabilized.

6. Calculation and Reporting

The fluoridation rate of the atmosphere is calculated as follows:

$$\text{Fluoridation rate} \left(\mu\text{g F}^- / 100 \text{ cm}^2 / 30 \text{ days} \right) = \frac{\mu\text{g F/ml} \times 50 \times 30 \times a}{b}$$

Where:

a = number of days of candle exposure

b = correction factor for 100 cm²

50 = volume of buffer solution (ml)

30 = fluoridation rate normalized for 30 days

7. Precision and Accuracy

The standard deviation is 0.034 at a concentration of 0.5 mg/l fluoride. Accuracy data is not yet available.

8. Bibliography

- 8.1. Adams, D.F. (1961). A quantitative study of the limed filter paper technique for fluorine air pollution studies. *International Journal of Air and Water Pollution*, 4: 247-255.
- 8.2. Adams, D.F. (1957). Further applications of the limed filter paper technique in fluorine air pollution studies. *Journal of Air and Water Pollution* 7: 88-91.
- 8.3. Environment Canada. (1972). National Inventory of Sources and Emissions of Fluoride. Report APCD 75-7, Abstract, (i).
- 8.4. Miller, V.L., Allmendinger, D.F., Johnson, F. and Polley, D. (1953). Limed papers and indicator plants in fluorine air pollution investigations. *Journal of Agriculture and Food Chemistry*, 1: 526-529.
- 8.5. Robinson, E. (1957). Determining fluoride air concentrations by exposing limed filter paper. *American Industrial Hygiene Association*, 18: 145-148.

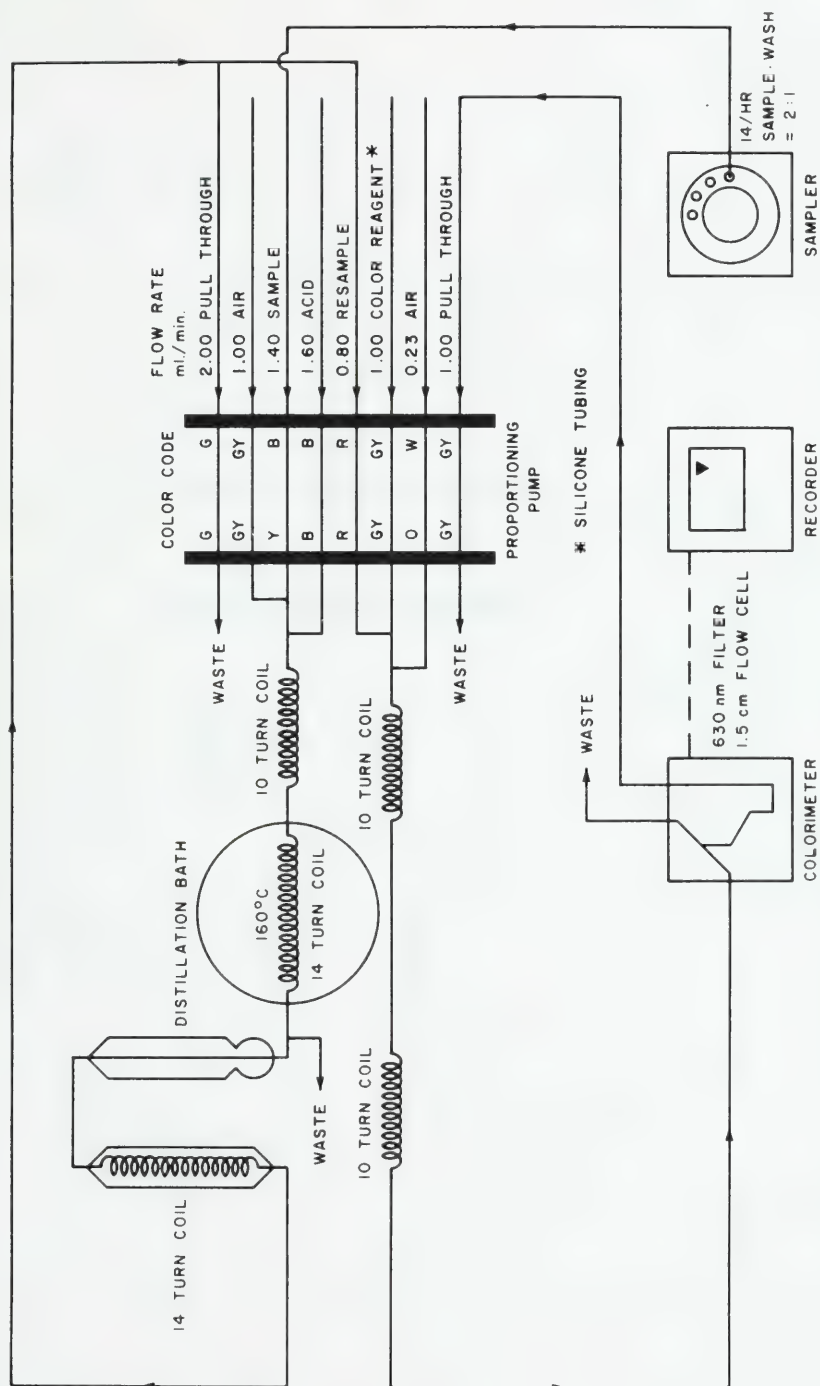


FIGURE 1 — AUTOANALYZER II SYSTEM FOR FLUORIDE DETERMINATION ON WATER SAMPLES

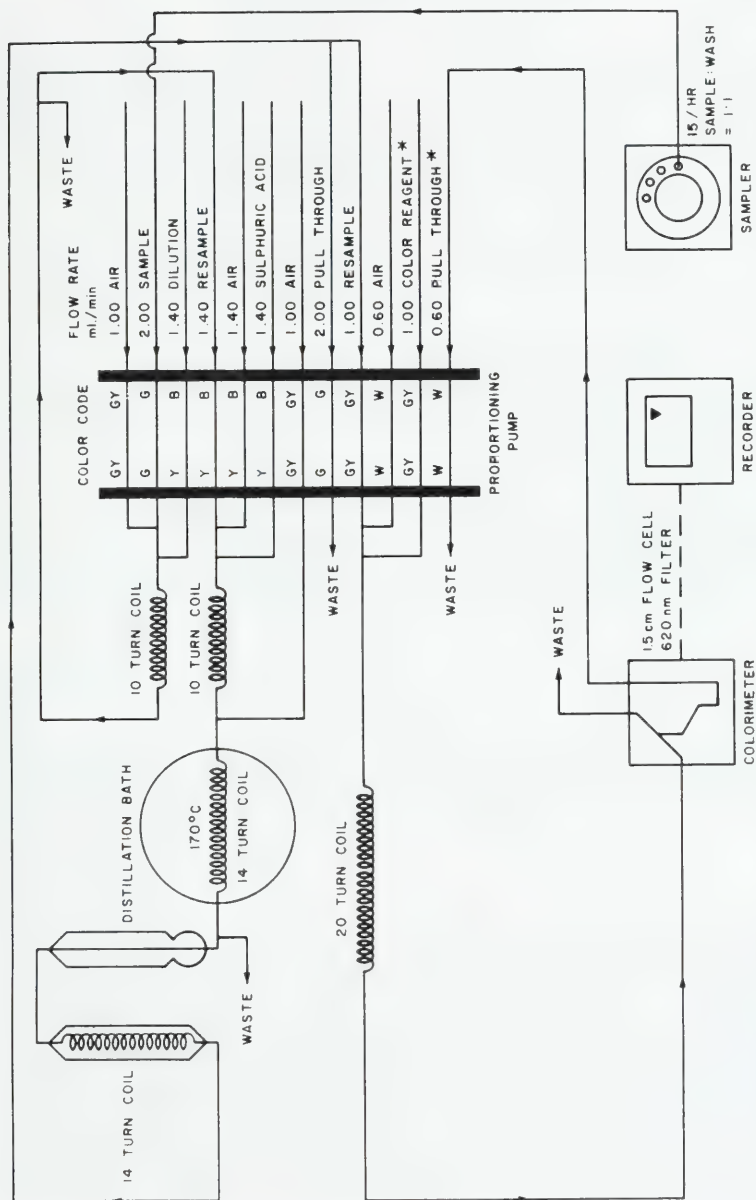


FIGURE 2 — AUTOANALYZER II SYSTEM FOR FLUORIDE DETERMINATION IN VEGETATION SAMPLES

THE DETERMINATION OF HARDNESS

Water hardness is due mainly to the presence of calcium and magnesium salts in solution. Other cations causing hardness, such as aluminum, iron, manganese, strontium and zinc, are not usually present in natural waters in sufficient concentrations to significantly affect the hardness. As a matter of convenience, hardness is reported as calcium carbonate (CaCO_3). Thus, the measured hardness, however caused, equals the hardness which would be produced by the quoted concentration of calcium carbonate. Theoretically, neither calcium nor carbonate need be present, but usually a significant portion of the hardness in natural waters is due to calcium.

The fraction of the total hardness accounted for by carbonate and bicarbonate salts in solution is called carbonate or temporary hardness and the remainder is noncarbonate or permanent hardness.

Disadvantages of hard water include excessive soap and detergent consumption and calcium and magnesium deposition at elevated temperatures forming a hard, heat insulating scale. This deposition reduces the life of hot water heaters, dish washers, washing machines, irons and other appliances which use hot water. Softer waters are somewhat more corrosive to metal plumbing and fixtures than hard waters.

The hardness of water varies widely throughout Ontario. In the Great Lakes system hardness increases downstream, from 50 mg/l in Lake Superior to 140 mg/l in Lake Ontario. While some unique ground waters in Ontario have hardness levels as high as 2,000 mg/l, levels in most well waters are less than 300 mg/l. Surface waters on the Precambrian Shield are commonly soft, having hardness levels of 40-75 mg/l, with some areas ranging as low as 10 mg/l.

Sample Handling and Preservation

Water

Samples containing large amounts of carbon dioxide may be unstable since a loss of carbon dioxide gas may cause precipitation of hardness salts. This loss may be minimized by filling the sample container to the neck and keeping it tightly closed and refrigerated (not frozen) until the test is performed.

Selection of Method

Method A, a manual titrimetric method, is routinely used on drinking water samples in the regional laboratories and consists of a titration with the chelating agent, ethylenediamine tetraacetic acid (EDTA), using Eriochrome Black T as indicator. Method B, a semi-automated titrimetric method is similar to Method A but samples are automatically titrated. This method is currently used in the Ministry laboratory in Toronto. A total hardness can also be derived by multiplying the calcium concentration as determined complexometrically or by atomic absorption by 2.49 and multiplying the magnesium concentration by 4.12 to give their equivalent calcium carbonate concentrations. The summation of these values yields the hardness concentration which is reported as calculated hardness. This method is used for routine hardness determinations on river and lake samples. For a detailed description of Calcium and Magnesium see sections on the Determination of Calcium, Determination of Magnesium and Determination of Metals.

HARDNESS

Manual Titrimetric Method A

SUMMARY

Matrix.	This method is used routinely on drinking water samples.
Substance determined.	The test measures dissolved cations which contribute to the hardness. If solid particles of hardness producing substances (such as precipitated calcium carbonate) are present in the sample they are not included in the determination as the pH chosen for the analysis maintains these salts in an insoluble state.
Interpretation of results.	Conventionally, hardness is reported in calcium carbonate (CaCO_3) units. Calcium and magnesium bicarbonates are the predominant hardness compounds present in most waters. In rare cases other trace metals are present in a large enough quantity to contribute to the hardness determination.
Principle of method.	An aliquot of sample, buffered at a high pH, is manually titrated with standard 0.01M ethylenediamine tetraacetic acid (EDTA), a chelating agent, to an endpoint indicated by Eriochrome Black T. As magnesium must be present to give a suitable endpoint, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer.
Time required for analysis.	Approximately 5 minutes are required for a single analysis. On a routine basis, about 80 tests can be performed in a day.
Range of application.	2 - 300 mg/l. Higher levels are measured by sample dilution.
Standard deviation.	2 mg/l in the range 2-300 mg/l.
Accuracy.	1% at the 200 mg/l level.
Detection criteria.	2 mg/l.
Interferences and shortcomings.	Interferences by some metal ions may cause fading or indistinct endpoints. This interference is reduced by the addition of appropriate inhibitors to the sample prior to titration with EDTA.

Minimum volume of sample.	125 ml.
Preservation and sample container.	Polyethylene or glass containers are suitable. With samples containing large amounts of carbon dioxide the glass containers must be filled to capacity and tightly capped to prevent loss of carbon dioxide gas. Refrigeration also reduces sample deterioration.
Safety considerations.	Buffer solution preparation should be carried out in a fume hood due to the heat generated and the objectionable odor involved. The concentrated ammonium hydroxide used to prepare the buffer must be handled with care to prevent burns. Extreme care should be taken when using the sodium cyanide inhibitor. Sample solutions containing this inhibitor should be treated by chlorination, then flushed down the drain with large quantities of water. The areas must be free of acids (this includes the drains) because acids liberate volatile HCN which is extremely poisonous.

HARDNESS

Manual Titrimetric Method A

1. Introduction

Eriochrome Black T is added to a solution containing hardness salts and buffered to pH 10. The resulting wine red colored solution is then titrated with EDTA to a blue endpoint. When the endpoint is reached all the calcium and magnesium has been complexed. Magnesium must be present to yield a satisfactory endpoint and, therefore, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer. This introduces sufficient magnesium and at the same time obviates a blank correction.

The sharpness of the endpoint increases with increasing pH, however, at an increased pH there is a danger of calcium carbonate or magnesium hydroxide precipitation and changes in the dye color. The pH value of 10.0 ± 0.1 recommended in this procedure is a satisfactory compromise. A 2 minute limit is set for the period of titration in order to minimize calcium carbonate precipitation.

2. Interferences and Shortcomings

Fading or indistinct endpoints can usually be alleviated by the use of inhibitor solutions. The hydroxylamine inhibitor is effective in minimizing interferences due to aluminum, copper, iron or manganese. Sodium cyanide, used as a last resort, will also control endpoint problems due to cobalt or nickel and higher levels of copper and iron. Samples failing to respond to these inhibitors are classed as unsuitable for the titrimetric hardness determination.

Calcium carbonate precipitation can also be minimized by repeating the test, adding up to 90% of the needed titrant before the buffer is introduced. In extreme cases, the sample may be acidified and stirred for a few minutes to eliminate the carbonates, then adjusted with buffer to a pH of 10.0 ± 0.1 , using a pH meter.

3. Apparatus

- 3.1 20 or 25 ml burette (preferably with automatic refill).
- 3.2 Magnetic stirrer and 2.5 cm (1 inch) spinbars (5).
- 3.3 250 ml flat bottom white porcelain casseroles (20).
- 3.4 pH meter, with pH division of 0.1 pH units over the range of 0-14 pH.
- 3.5 Reagent bottles, in appropriate sizes, clear, with ground glass stoppers.
- 3.6 Oxford auto pipettor.
- 3.7 Volumetric pipettes, 5,10,25,50,100 ml.

- 3.8 Volumetric flask, 1 liter.
- 3.9 Polyethylene Carboy, 20 liters.

4. Reagents

- 4.1 Calcium carbonate anhydrous (CaCO_3), reagent grade, powder.
- 4.2 Ethylenediamine tetraacetic acid, disodium salt ($(\text{CH}_2\text{N}(\text{CH}_2\text{COOH})\cdot\text{CH}_2\text{COONa})_2\cdot 2\text{H}_2\text{O}$)
- 4.3 Eriochrome Black T, reagent grade, powder.
- 4.4 Sodium chloride (NaCl), reagent grade, powder.
- 4.5 Sulphuric acid (H_2SO_4), concentrated, reagent grade.
- 4.6 Ethylenediamine tetraacetic acid disodium, magnesium salt ($\text{Na}_2\text{Mg EDTA}$) reagent grade, powder.
- 4.7 Ethanolamine ($\text{CH}_2(\text{OH})\cdot\text{CH}_2\cdot\text{NH}_2$), reagent grade.
- 4.8 Ammonium chloride (NH_4Cl) reagent grade, powder.
- 4.9 Ammonium hydroxide (NH_4OH), reagent grade, concentrated.
- 4.10 Methyl red indicator solution.
- 4.11 Sodium cyanide (NaCN) reagent grade, powder.
- 4.12 Hydroxylamine hydrochloride (OHNH_2Cl) reagent grade.
- 4.13 Household bleach, 5.25% available chlorine.
- 4.14 **Standard Calcium Carbonate Solution (1000 mg/l)**

Weight 1.000 g anhydrous calcium carbonate into a 500 ml Erlenmeyer flask. Slowly add 1/l, (v/v) hydrochloric acid until all the calcium carbonate has dissolved. Add 200 ml distilled water and boil for a few minutes to expel carbon dioxide. Cool, add a few drops of methyl red indicator and adjust to the intermediate orange color by adding 3N ammonium hydroxide or 1/l (v/v) hydrochloric acid as required. Transfer to a 1 liter volumetric flask and fill to the mark with distilled water. Store the solution in a glass reagent bottle.

4.15 Eriochrome Black T Indicator Powder

Mix together (in mortar with pestle) 1.0 g dye and 200 g sodium chloride to prepare a dry powder mixture. Commercial varieties of this dye include: "Eriochrome Black T" (Geigy); "Solochrome Black W DFA" (C.I.E.) and "Pontachrome Black" (Dupont).

4.16 Hydrochloric Acid - Ethanolamine - Magnesium EDTA Buffer Solution

Dilute 110 ml concentrated hydrochloric acid to 800 ml with distilled water in a 2 liter volumetric flask. In the fumehood carefully add 620 ml ethanolamine and 10 g Mg EDTA and dilute to 2 liters with distilled water. Store the solution in a glass reagent bottle.

NOTE: The pH of this buffer should be checked and adjusted to pH 10.0 by further addition of concentrated hydrochloric acid, if necessary.

4.17 Ammonium Chloride - Ammonium Hydroxide - Magnesium EDTA Buffer Solution

NOTE: Prepare this reagent in a fumehood.

Dissolve 135 g ammonium chloride in 1140 ml concentrated ammonium hydroxide, add 10.0 g Mg EDTA and dilute to 2 liters with distilled water.

NOTE: Although this is the preferred buffer, the volatility of the ammonia makes possible the contamination of other tests being performed in the same laboratory thus limiting its use.

4.18 Hydroxylamine Hydrochloride Inhibitor Solution (10%)

Dissolve 100 g hydroxylamine hydrochloride in 1 liter distilled water.

4.19 Standard EDTA Titrant (0.01 M) (equal to 1 mg/l CaCO_3)

Dissolve 74.46 g disodium ethylenediamine tetraacetic acid dihydrate (EDTA) in distilled water and dilute to 20 liters. Standardize against standard calcium solution.

4.19.1 Standardization

Pipette 10.00 ml standard calcium carbonate solution into the casserole and add 2 ml of buffer solution, followed by an appropriate amount of dry-powder indicator. While stirring, titrate, with standard EDTA titrant to the Eriochrome Black T endpoint. The endpoint is reached when a blue color persists. Repeat this procedure three times and calculate the average molarity of the titrant. If necessary, adjust the concentration of the titrant by adding distilled water or EDTA disodium salt, and restandardize as before until the solution is exactly 0.0100 M (1.00 ml = 1.00 mg calcium carbonate).

4.20 Quality Control Stock Solution

Weigh 4.49475 ± 0.00005 g anhydrous calcium carbonate into a 500 ml volumetric flask. Add, dropwise, 1/1 (v/v) hydrochloric acid until all the calcium carbonate has dissolved. Make volume to 200 ml and boil for a few minutes to expel carbon dioxide. Cool, add 1 - 2 drops methyl red indicator and adjust color to intermediate orange by adding 3N ammonium hydroxide or 1/1 (v/v) hydrochloric acid. Transfer to 2 liter volumetric flask. Add 0.38159 ± 0.00005 g magnesium sulphate. Dissolve and make to 2 liters with distilled water. Add 1 - 2 ml chloroform.

4.21 Quality Control Working Solutions

QC-A: Dilute 50 ml quality control stock solution to 2 liters with distilled water to give a hardness concentration of 232.5 mg/l as CaCO_3 .

QC-B: Dilute 20 ml quality control stock solution to 2 liters with distilled water to give a hardness concentration of 93 mg/l as CaCO_3 .

Discard stock solution.

5. Procedure

- 5.1 Select a sample volume, usually 50 ml, which will require a titrant volume ranging from a low of 3 ml to a maximum of 15 ml. The lower volume limit will ensure a minimum loss of precision caused by the measurement of small volumes.

Where the conductivity of the sample is known, the following guide often applies:

<u>Conductivity</u>	<u>Aliquot</u>
< 150 $\mu\text{mho/cm}$	100 ml
150-600 $\mu\text{mho/cm}$	50 ml
600-1200 $\mu\text{mho/cm}$	25 ml
> 1200 $\mu\text{mho/cm}$	an aliquot to give a titration greater than 7.5 ml

Aliquots equivalent to less than 5 ml of original sample must be obtained by prior dilution in a volumetric flask.

- 5.2 Pipette a sample aliquot into a casserole with spinbar and bring volume up to 50 ml where aliquot is less. Place casserole on a magnetic stirrer.
- 5.3 Add 2 ml of buffer solution.
- 5.4 Add an appropriate amount of indicator.
- 5.5 Add EDTA titrant slowly, with continuous stirring, until the reddish tinge disappears from the solution. The color of the solution at the endpoint is blue. Interfering substances may prevent exact matches between the blue endpoint color of blanks and samples.

NOTE: After the addition of buffer, do not extend the titration beyond 2 minutes.

- 5.6 Record the amount of titrant used.
- 5.7 **Use of Inhibitors for Control of Interferences**

Two inhibitors are used, the choice depending on the severity of the interference.

- 5.7.1 10% hydroxylamine hydrochloride is used where the interference is minimal. Add the reagent after step 5.2, and allow the solution to stand one minute before proceeding.
- 5.7.2 Sodium cyanide powder is used for severe interferences. If used the following procedure must be strictly obeyed:
- 5.7.2.1 After step 5.3, ensure that the solution is basic (pH paper).
- 5.7.2.2 Add approximately 0.25 g dry powder sodium cyanide using a scoop.

- NOTE:** Treat this potent poison with the utmost caution.
- 5.7.2.3. Proceed with the titration.
 - 5.7.2.4. Add 5 ml household bleach (or 20 ml for each gram of sodium cyanide) and stir the solution for a few seconds.
 - 5.7.2.5. Discard the solution directly into a sink drain; follow with a copious running water rinse.

6. Calculation and Reporting

Hardness is calculated using the following equation:

$$\text{Hardness as mg/l CaCO}_3 = \frac{a \times b \times 1,000}{\text{ml of sample}}$$

Where:

a = ml titration for sample

b = mg CaCO₃ equivalent to 1.00 ml EDTA

Report to the nearest 1 mg/l in the range 2-600 mg/l, and to two significant figures above 600 mg/l.

7. Precision and Accuracy

The standard deviation is 2 mg/l based on a large number of samples analyzed in duplicate, and is independent of concentration in the range 2-300 mg/l.

8. Bibliography

- 8.1 American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater, 13th ed. APHA, Washington, D.C.
- 8.2 U.S. Geological Survey (1970). Study and interpretation of the chemical characteristics of water. Geological Survey Water Supply Paper No. 1473, U.S. Department of the Interior 363 p.

HARDNESS

Semi-Automated Titrimetric Method B

SUMMARY

Matrix.	This method is used routinely on drinking water samples.
Substance determined.	The test measures dissolved cations which contribute to the hardness. If solid particles of hardness producing substances (such as precipitated calcium carbonate) are present in the sample they are not included in the determination as the pH chosen for the analysis maintains these salts in an insoluble state.
Interpretation of results.	Conventionally, hardness is reported in calcium carbonate (CaCO_3) units. Calcium and magnesium bicarbonates are the predominant hardness compounds present in most waters. In rare cases other trace metals are present in a large enough quantity to contribute to the hardness determination.
Principle of method.	An aliquot of sample is manually pipetted, made up to 50 ml and buffer solution added. Eriochrome Black T indicator is automatically added and samples are automatically titrated with EDTA to a blue endpoint.
Time required for analysis.	Approximately 5 minutes are required for a single analysis. On a routine basis, about 80 tests can be performed in a day.
Range of application.	1.4 - 300 mg/l calcium carbonate. Higher levels are measured by sample dilution.
Standard deviation.	Standard deviations based on within-run duplicate samples are: 0.82 for 0-20% of range; 0.74 for 20-50% of range and 0.90 for 50-100% of range.
Accuracy.	Recoveries of two Quality Control standards were 100% and 101% respectively.
Detection criteria.	1.35 mg/l.
Interferences and shortcomings.	Interferences by some metal ions may cause fading or indistinct endpoints. This interference is reduced by the addition of appropriate inhibitors to the sample prior to titration with EDTA.

**Minimum volume
of sample.**

125 ml.

**Preservation and
sample container.**

Polyethylene or glass containers are suitable. With samples containing large amounts of carbon dioxide the glass containers must be filled to capacity and tightly capped to prevent loss of carbon dioxide gas. Refrigeration also reduces sample deterioration.

**Safety
considerations.**

Buffer solution preparation should be carried out in a fume hood due to the heat generated and the objectionable odor involved. The concentrated ammonium hydroxide used to prepare the buffer must be handled with care to prevent burns. Extreme care should be taken when using the sodium cyanide inhibitor. Sample solutions containing this inhibitor should be treated by chlorination, then flushed down the drain with large quantities of water. The areas must be free of acids (this includes the drains) because acids liberate volatile HCN which is extremely poisonous.

HARDNESS

Semi-Automated Titrimetric Method B

1. Introduction

Eriochrome Black T is automatically added to a sample solution containing hardness salts and buffered to pH 10. The resulting wine red colored solution is then titrated with EDTA to a blue endpoint. When the endpoint is reached all the calcium and magnesium has been complexed. Magnesium must be present to yield a satisfactory endpoint and, therefore, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer. This introduces sufficient magnesium and at the same time obviates a blank correction.

The sharpness of the endpoint increases with increasing pH, however, at an increased pH there is a danger of calcium carbonate or magnesium hydroxide precipitation and changes in the dye color. The pH value of 10.0 ± 0.1 recommended in this procedure is a satisfactory compromise. A 2 minute limit is set for the period of titration in order to minimize calcium carbonate precipitation.

2. Interferences and Shortcomings

Fading or indistinct endpoints can usually be alleviated by the use of inhibitor solutions. The hydroxylamine inhibitor present in the buffer is effective in minimizing interferences due to aluminum, copper, iron or manganese. Sodium cyanide, used as a last resort, will also control endpoint problems due to cobalt or nickel and higher levels of copper and iron. Samples failing to respond to these inhibitors are classed as unsuitable for the titrimetric hardness determination.

3. Apparatus

- 3.1. Radiometer AutoBurette ABU 1c, 25 ml barrel volume.
- 3.2. Radiometer Phototitration PMT 1.
- 3.3. Clear glass beakers 250 ml.
- 3.4. Reagent bottles, in appropriate sizes, clear, with ground glass stoppers.
- 3.5. Oxford auto pipettor.
- 3.6. Volumetric pipettes, 5,10,25,50,100 ml.
- 3.7. Volumetric flask, 1 liter.
- 3.8. Polyethylene Carboy, 20 liters.

4. Reagents

- 4.1. Calcium carbonate, anhydrous (CaCO_3), reagent grade, powder.
- 4.2. Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), reagent grade crystals.
- 4.3. Ethylenediamine tetraacetic acid, disodium salt
($(\text{CH}_2\text{N}(\text{CH}_2\text{COOH})\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$)
- 4.4. Eriochrome Black T, reagent grade, powder.
- 4.5. Ethylenediamine tetraacetic acid disodium magnesium salt ($\text{Na}_2\text{Mg EDTA}$), reagent grade, powder.
- 4.6. Ammonium chloride (NH_4Cl), reagent grade powder.
- 4.7. Ammonium hydroxide (NH_4OH), reagent grade, concentrated.
- 4.8. Methyl red indicator solution.
- 4.9. Sodium cyanide (NaCN), reagent grade, powder.
- 4.10. Hydroxylamine hydrochloride (OHNH_2Cl), reagent grade.
- 4.11. Household bleach, 5.25% available chlorine.
- 4.12. Chloroform (CHCl_3), reagent grade.

4.13. Standard Calcium Carbonate Solution (1000 mg/l)

Weigh 2.00000 g ± 0.00005 g anhydrous calcium carbonate into a 500 ml Erlenmeyer flask. Add, dropwise, 1/1 (v/v) hydrochloric acid until all the calcium carbonate has dissolved. Add 200 ml distilled water and boil for a few minutes to expel carbon dioxide. Cool, add a few drops of methyl red indicator and adjust to the intermediate orange color by adding 3N ammonium hydroxide or 1/1 (v/v) hydrochloric acid as required. Transfer to a 2 liter volumetric flask and fill to the mark with distilled water. Add 1 - 2 ml chloroform. Store the solution in a glass reagent bottle.

4.14. Eriochrome Black T Indicator Solution

Dissolve 0.10 g Eriochrome Black T indicator in 500 ml distilled water. Commercial varieties of this dye include: Eriochrome Black T (Geigy); Solochrome Black W DFA (CIE) and Pontachrome Black (Dupont).

4.15. Hydrochloric Acid (1/1 v/v)

Dilute 50 ml concentrated hydrochloric acid to 1 liter with distilled water.

4.16. Methyl Red Solution

Dissolve 0.10 g methyl red sodium salt in distilled water and dilute to 100 ml.

4.17. Ammonium Hydroxide Solution (3N)

Dilute 20 ml concentrated ammonium hydroxide to 100 ml with distilled water.

4.18. Buffer Solution

Dissolve 270.4 g ammonium chloride in 2,300 ml concentrated ammonium hydroxide. Add 20.0 g sodium-magnesium EDTA and 4.0 g hydroxylamine hydrochloride and dilute to 4 liters with distilled water.

Prepare this reagent in the fumehood.

NOTE: Although this is the preferred buffer, the volatility of the ammonia makes possible the contamination of other tests being performed in the same laboratory thus limiting its use.

4.19. Standard EDTA Titrant (0.01M) (equal to 1 mg/l as CaCO_3)

Dissolve 74.46 g disodium ethylenediamine tetraacetic acid dihydrate (EDTA) in distilled water and dilute to 20 liters. Standardize against standard calcium solution.

4.19.1. Standardization

Pipette 10.00 ml standard calcium carbonate solution into the casserole and add 2 ml buffer solution, followed by an appropriate amount of dry-powder indicator. While stirring, titrate, with standard EDTA titrant to the Eriochrome Black T endpoint. The endpoint is reached when a blue color persists. Repeat this procedure three times and calculate the average molarity of the titrant. If necessary, adjust the concentration of the titrant by adding distilled water or EDTA disodium salt, and restandardize as before until the solution is exactly 0.0100M (1.00 ml = 1.00 mg calcium carbonate).

4.20. Quality Control Stock Solution

Weigh 4.49475 ± 0.00005 g anhydrous calcium carbonate into a 500 ml volumetric flask. Add, dropwise, 1/1 (v/v) hydrochloric acid until all the calcium carbonate has dissolved. Make volume to 200 ml and boil for a few minutes to expel carbon dioxide. Cool, add 1 - 2 drops methyl red indicator and adjust color to intermediate orange by adding 3N ammonium hydroxide or 1/1 (v/v) hydrochloric acid. Transfer to 2 liter volumetric flask. Add 0.38159 ± 0.00005 g magnesium sulphate. Dissolve and make to 2 liters with distilled water. Add 1 - 2 ml chloroform.

4.21. Quality Control Working Solutions

QC-A: Dilute 50 ml quality control stock solution to 2 liters with distilled water to give a hardness concentration of 232.5 mg/l as CaCO_3 .

QC-B: Dilute 20 ml quality control stock solution to 2 liters with distilled water to give a hardness concentration of 93 mg/l as CaCO_3 .

Discard stock solution.

5. Procedure

- 5.1 Select a sample volume, usually 50 ml, which will require a titrant volume ranging from a low of 3 ml to a maximum of 15 ml. The lower volume limit will ensure a minimum loss of precision caused by the measurement of small volumes.

Where the conductivity of the sample is known, the following guide often applies:

<u>Conductivity</u>	<u>Aliquot</u>
< 150 $\mu\text{mho/cm}$	100 ml
150-600 $\mu\text{mho/cm}$	50 ml
600-1200 $\mu\text{mho/cm}$	25 ml
> 1200 $\mu\text{mho/cm}$	an aliquot to give a titration greater than 7.5 ml

Aliquots equivalent to less than 5 ml of original sample must be obtained by prior dilution in a volumetric flask.

- 5.2 Pipette a sample aliquot into a beaker. Make up to 50 ml where aliquot is less. Refill burette and turn to titration position.
- 5.3. Add 2 ml of buffer solution.
- 5.4. Place on phototitrator platform, raise until stirrer starts and press start button.
- 5.5. Record the amount of titrant used.
- 5.6. **Use of Inhibitors for Control of Interferences**

Two inhibitors are used, the choice depending on the severity of the interference.

- 5.6.1. 10% hydroxylamine is used where the interference is minimal. Add the reagent after step 5.2 and allow solution to stand one minute before proceeding.
- 5.6.2. Sodium cyanide powder is used for severe interferences. If used the following procedure must be strictly obeyed:
 - 5.6.2.1. After step 5.3, ensure that the solution is basic (pH paper).
 - 5.6.2.2. Add approximately 0.25 g dry powder sodium cyanide using a scoop.
NOTE: Treat this potent poison with the utmost caution.
 - 5.6.2.3. Proceed with the titration.
 - 5.6.2.4. Add 50 ml household bleach (or 20 ml for each gram of sodium cyanide) and stir the solution for a few seconds.
 - 5.6.2.5. Discard the solution directly into a sink drain; follow with a copious running water rinse.

6. Calculation and Reporting

Hardness is calculated using the following equation:

$$\text{Hardness as mg/l CaCO}_3 = \frac{a \times b \times 1,000}{\text{ml of sample}}$$

Where:

a = ml titration for sample

b = mg CaCO₃ equivalent to 1.00 ml EDTA

Report to the nearest 1 mg/l in the range 1-99 mg/l, and to 3 significant figures above 100 mg/l.

7. Precision and Accuracy

Standard deviations based on within-run duplicate samples are: 0.82 for 0 - 20% of range; 0.74 for 20 - 50% of range and 0.90 for 50 - 100% of range.

Recoveries of two Quality Control standards were 100% and 101% respectively.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater. 13th ed. APHA, Washington, D.C.
- 8.2. U.S. Geological Survey (1970). Study and interpretation of the chemical characteristics of water. Geological Survey Water Supply Paper No. 1473, U.S. Department of the Interior, 363 p.

THE DETERMINATION OF IRON

Iron is relatively common in the environment and enters the soil and aquatic systems through the weathering of igneous rocks such as iron ores, biotite micas and ferromagnesium silicates. Iron is also associated with sedimentary rocks such as siderite (iron carbonate).

The species of iron present in water are diverse and in a state of flux as a result of shifting chemical equilibria and biological activity. Samples may contain particulate iron or iron oxides embedded in silt or clay or as flakes of rust from pipes. Iron may be present as a colloid peptized by organic material, as dissolved undissociated complexes and hydrates, or as several hydrated forms of ferric or ferrous ions in solution.

In soil, iron occurs in the primary minerals as amorphous oxides, in an organically complexed form, and as ferrous and ferric ions in the soil solution. Iron salts rarely occur in significant amounts with the exception of acid soils where iron sulphates are present. The hydrolysis of iron in certain soil systems can result in some exchangeable iron adsorbed on clay surfaces.

Ferrous iron is relatively soluble and is present in this form in anaerobic soil and water systems. In waterlogged soils of this nature characteristic greenish and gray colors occur. Upon contact with dissolved oxygen and at alkaline pH's the ferrous iron rapidly oxidizes to the ferric forms. At a pH of 7 or greater, ferric iron is undissociated and exists as the relatively insoluble "hydrate" which readily forms a colloidal suspension and slowly precipitates from solution. A decrease in pH can resolubilize this suspension and at a pH below 5, ferric iron is significantly soluble.

In soil and water systems the ability of organic compounds to complex iron contributes greatly to its solubility. This process is important in soil systems as iron may be mobilized at pH levels as high as 8. Bacteria present in soil water supplies alter the dissolved iron content and/or the relative proportions of ferric/ferrous iron by ingestion and/or secretion. In some cases the ferric hydroxide accumulates on the surface of the colony, and when dislodged by flowing water, these gelatinous clumps present a nuisance problem in water distribution systems.

Although an essential plant nutrient, iron is present in vegetation in only trace amounts. Iron deficiency is most common in alkaline soils where iron is unavailable. Iron chelates in solution can be sprayed on the soil to overcome this problem.

Iron is of interest, especially in water and wastewater systems since concentrations of a few tenths of a mg/l can impart unpleasant taste to drinking water and stain plumbing fixtures. Iron is active in the storage and release mechanisms of phosphate in lake sediments and is used as a phosphate precipitant in nutrient removal sewage treatment processes.

Sample Handling and Preservation

Water

It is recommended that samples be collected in separate bottles for iron analysis only. These containers should be rinsed with dilute nitric acid prior to sampling and preserved with nitric acid at $\text{pH} \approx 2$ to prevent colloidal iron from adhering to container walls.

The form and concentration of iron in well or tap water samples may vary with the period and degree of flushing before and during sampling.

For soil and vegetation sampling see the Determination of Trace Metals.

Selection of Method

In the main laboratory surface and domestic water samples as well as sewage samples are analyzed by Method A, an autoclave-automated TPTZ colorimetric method.

Soils and vegetation samples are analyzed by atomic absorption spectrophotometry or by ICP. These methods are described in the Determination of Trace Metals.

TOTAL IRON

Autoclave-Automated TPTZ Colorimetric Method A

SUMMARY

Matrix.	This method is used on drinking waters, sewages and river and lake samples.
Substance determined.	All iron species present in the sample are determined as Fe. These include particulate and colloidal iron, dissolved undissociated iron complexes and hydrates as well as ferrous and ferric ions in solution.
Interpretation of results.	Results are reported in mg/l iron as Fe.
Principle of method.	An aliquot of sample is treated with a digestant and autoclaved to bring all the iron present into the ferrous state. A portion of the digested sample is automatically introduced into an AutoAnalyzer where it is proportioned with hydroxylamine hydrochloride and TPTZ color reagent, and buffered to cover the color intensity range from pH 3.5 to pH 5.8. The color intensity, proportional to the iron content is measured at 600 nm and displayed as a peak on a chart recorder. The concentration is determined by comparing the sample peak with peaks obtained from a series of undigested known standards.
Time required for analysis.	Approximately 150 analyses may be completed per day.
Range of application.	0.02 - 1.0 mg/l Fe on undiluted river and lake samples.
Standard deviation.	Rivers and lakes: 0.014 for 0 - 20% of the range; 0.028 for 20 - 50% of the range and 0.025 for 50 - 100% of the range.
Accuracy.	Accuracy is controlled by 2 independently prepared long-term standards QC-A and QC-B in such a way that $(A + B)$ and $(A - B)$ do not vary by more than 2 standard deviations from their long-term means. These control limits are 0.03 mg/l Fe for river and lake samples.

Detection Criteria	0.02 mg/l Fe.
Interferences and shortcomings.	<p>There are no significant interferences found in normal ground and surface water samples.</p> <p>NOTE: The black bakelite caps normally supplied with the culture tubes used for digestion, are unsuitable due to iron contamination and should be replaced with a size 24 polypropylene screw cap.</p>
Minimum sample size.	75 ml for samples with up to 1.0 mg/l Fe, less for samples with higher concentrations.
Preservation and sample container.	Glass or polyethylene bottles rinsed with nitric acid are suitable. Ground and surface water samples are normally submitted without preservation. Nitric acid may be added to produce a pH \approx 2 in order to prevent colloidal iron from adhering to the container walls.
Safety considerations.	Normal laboratory safety procedures should be followed at all times, particularly those pertaining to concentrated acids. The operating instructions for the autoclave must be strictly followed. Sulphuric acid is used in the digestion solution in order to minimize corrosion effects within the autoclave.

TOTAL IRON

Autoclave-Automated TPTZ Colorimetric Method A

1. Introduction

Iron is solubilized by autoclaving in an acid-reducing media at 121 °C for 40 min. and then determined colorimetrically using a Technicon AutoAnalyzer II by formation of the ferrous-2,4,6-Tri-(2'pyridyl)-1,3,5,-triazine (TPTZ) complex. The violet ferrous-TPTZ complex is buffered into the pH range of 3.4 to 5.8 by the incorporation of a sodium acetate-acetic acid buffer system. The colored complex formed obeys Beer's law over the working range.

2. Interferences and Shortcomings

There are no significant interferences found in normal groundwater or surface water samples.

3. Apparatus

3.1. AutoAnalyzer AAII system consisting of the following modules:

- 3.1.1. Sampler; 100 or 200 place model.
- 3.1.2. Proportioning pump
- 3.1.3. Colorimeter equipped with 600 nm filters and a 5 cm flow cell.
- 3.1.4. Voltage regulator for above colorimeter.
- 3.1.5. Chart recorder.

3.2. Manifold pump tubing and glassware assembled as shown in Figure 1.

3.3. Culture tubes, 25 mm x 150 mm with Nalgene size 24 polypropylene screw caps.

3.4. Assorted volumetric and transfer pipettes.

3.5. Racks to hold 40 culture tubes each.

3.6. Dilution tubes.

3.7. Oxford automatic pipettor, 1 - 10.0 ml delivery capacity.

4. Reagents

All reagents including the deionized distilled water must be low in iron content. The iron content of digested blanks must be less than 0.01 mg/l Fe.

- 4.1. Hydrochloric acid, (HCl), Aristar grade, concentrated.
- 4.2. 2,4,6-Tri-(2'-pyridyl)-1,3,5-triazine (TPTZ) ($C_{18}H_{12}N_6 \cdot H_2O$), reagent grade crystals.
- 4.3. Ferrous ammonium sulphate hexahydrate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$), reagent grade crystals.
- 4.4. Hydroxylamine hydrochloride, ($NH_2OH \cdot HCl$), reagent grade, crystals (for surface water samples only).
- 4.5. Sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$), reagent grade crystals.
- 4.6. Acetic acid, glacial, (CH_3COOH), reagent grade.
- 4.7. Sulphuric acid, (H_2SO_4), analytical reagent, concentrated.
- 4.8. Stock iron solution, 1000 mg/l Fe, BDH analytical reagent.
- 4.9. Potassium ferricyanide, ($K_3Fe(CN)_6$), analytical reagent, crystals.

NOTE: Due to the use of concentrated acids, considerable care is required in the preparation of reagents, standards, and during the digestion step.

4.10. TPTZ Solution

In a volumetric flask dissolve 0.1500 g TPTZ reagent in 2 ml concentrated hydrochloric acid and dilute to 1 liter with deionized, distilled water.

4.11. Hydroxylamine Hydrochloride Solution

In a volumetric flask dissolve 7.0 g hydroxylamine hydrochloride in deionized, distilled water and dilute to 1 liter.

4.12. Sodium Acetate Buffer

Dissolve 870 g sodium acetate trihydrate in deionized, distilled water. Carefully add 230 ml glacial acetic acid and dilute to 2 liters.

4.13. Digestion Acid

Dissolve 120.0 g hydroxylamine hydrochloride in deionized, distilled water. Carefully add 800 ml concentrated sulphuric acid and dilute to 4 liters.

4.14. Wash Water

Dilute 50 ml concentrated sulphuric acid to 4 liters with deionized, distilled water.

4.15. Blank Solution

Dilute 62 ml digestion acid to 1 liter with deionized distilled water.

4.16. Iron Recovery Stock Solution 1000 mg/l (1000 mg/l as Fe)

In a volumetric flask dissolve 5.8956 g potassium ferricyanide in deionized, distilled water and dilute to 1 liter.

4.17. Standard Iron Recovery Solution (20 mg/l as Fe)

In a volumetric flask dilute 20 ml recovery stock solution to 1 liter with deionized, distilled water.

4.18. Working Recovery Solutions

Two recovery solutions are prepared by diluting aliquots of the standard iron recovery solution with deionized, distilled water using volumetric pipetts and flasks: 10.0 ml/l and 40.0 ml/l give standards with iron concentrations of 0.20 and 0.80 mg/l Fe respectively.

4.19. Iron Stock Solution (1000 mg/l as Fe)

In a volumetric flask dissolve 7.0217 g ferrous ammonium sulphate hexahydrate in deionized, distilled water; add 1 ml concentrated sulphuric acid and dilute to 1 liter with deionized, distilled water.

4.20. Standard Iron Solution (20 mg/l as Fe)

In a volumetric flask dilute 20 ml iron stock solution in deionized distilled water and dilute to 1 liter.

4.21. Iron Working Standards and In-run Sensitivity Checks

Low: In a volumetric flask add 10 ml standard iron solution to deionized, distilled water containing 62 ml digestion acid and dilute to 1 liter. As this standard already contains the appropriate concentration of digestion acid, its effective iron concentration is 0.21 mg/l Fe.

High: In a volumetric flask add 40 ml standard iron solution to deionized, distilled water containing 62 ml digestion acid and dilute to 1 liter. As this standard already contains the appropriate concentration of digestion acid, its effective iron concentration is 0.85 mg/l Fe.

4.22. Quality Control Standard Solution (80 mg/l as Fe)

In a volumetric flask, dilute 80 ml of reagent 4.8 to 1 liter with deionized, distilled water.

4.23. Quality Control Working Solutions:

QC-A (0.60 mg/l Fe): In a volumetric flask dilute 15.0 ml quality control standard solution to 2 liters with deionized, distilled water. Then add 133 ml digestion acid, and mix.

QC-B (0.40 mg/l Fe): In a volumetric flask dilute 10.0 ml quality control standard solution to 2 liters with deionized, distilled water. Then add 133 ml digestion acid, and mix.

5. Procedure

5.1. Pipet 30.0 ml a well shaken sample into a culture tube.

5.2. Add 2.0 ml of digestion acid, tighten screw cap and shake tube.

- 5.3. Similarly, set up 2 deionized distilled water blanks and 6 recovery standards: 2 low (0.20 mg/l Fe), 4 high (0.80 mg/l Fe).
- 5.4. Autoclave blanks, recovery standards and samples at 121⁰ C to 127⁰ C for 40 min.
NOTE: The manufacture's procedure for operating the autoclave on the liquid cycle must be strictly followed. For safety, wait 15 min. before removing racks of digested samples from the autoclave.
- 5.5. When samples have cooled to room temperature, visually inspect culture tubes for volume loss. Repeat any samples showing a volume loss greater than 3%.
- 5.6. Set up AutoAnalyzer as in Figure 1 using the following series of undigested standards, quality control solutions, digested and undigested blanks and recovery standards:
 0.85 STD; Bl; 0.85 STD; Bl; 0.21 STD; 0.85 STD; Bl; LTBl; QC-A; QC-B; Bl; 2 DIG Bl; Recovery STDS (0.20, 0.20, 0.80, 0.80, 0.80, 0.80); Bl.
- 5.7. Load drum with samples, sensitivity checks and blanks as follows:

 LOW; HIGH; Bl; 10 samples; Bl; 10 samples; LOW; HIGH; Bl; 10 samples . . .
 etc.
 Where: LOW = 0.21 mg/l as Fe
 HIGH = 0.85 mg/l as Fe
- 5.8. Read the concentration of iron directly by comparison of the sample peaks with the peaks of the known undigested standards using an appropriate calibration curve.

6. Calculation and Reporting

The iron concentration is calculated as follows:

$$\text{Fe in mg/l} = \text{reading} \times \text{dilution factor}$$

Results are reported to 3 significant figures if feasible.

7. Precision and Accuracy

Standard deviations for within-run duplicate samples are as follows:

Sample Type	Concentration	S _{ld}	S _{md}	S _{hd}
River and Lakes	0.01 - 1.0 mg/l	0.014	0.028	0.025

Where: S_{ld} = standard deviation for 0 - 20% of range S_{md} = standard deviation for 20 - 50% of range S_{hd} = standard deviation for 50 - 100% of range

Accuracy is controlled by 2 independently prepared long-term standards QC-A and QC-B at approximately 80% and 20% of range. Control is maintained in such a way

that $(A + B)$ and $A - B$ do not vary by more than 3 standard deviations from the long-term means. These control limits are 0.03 mg/l Fe.

8. Bibliography

- 8.1. Armstrong, F.A. (1957). The iron concentration of sea water. *Journal of the Marine Biology Association*. 36; 509.
- 8.2. Diehl, H., and Smith, G.F. (1965). The Iron Reagents: Bathophenanthroline, Bathophenanthroline-Disulfuric Acid, 2,4,6-Tripyridyl-s-triazine, Phenyl-2-pyridyl ketoxime. 2nd edition, G. Frederick Smith Chemical Co., Columbus, Ohio.
- 8.3. Dougan, W.K. and Wilson A.L. (1973). Absorptiometric determination of iron with TPTZ, *Water Treatment and Examination*. 22: 100.
- 8.4. Crowther, J. (1976). Absorptiometric Determination of Iron in Water Samples. Internal Report dated Mar. 29, 1976.
- 8.5. Crowther, J. (1978). Autoclave Digestion Procedure for the Determination of Total Iron Content of Waters. *Anal. Chem.* 50:659.

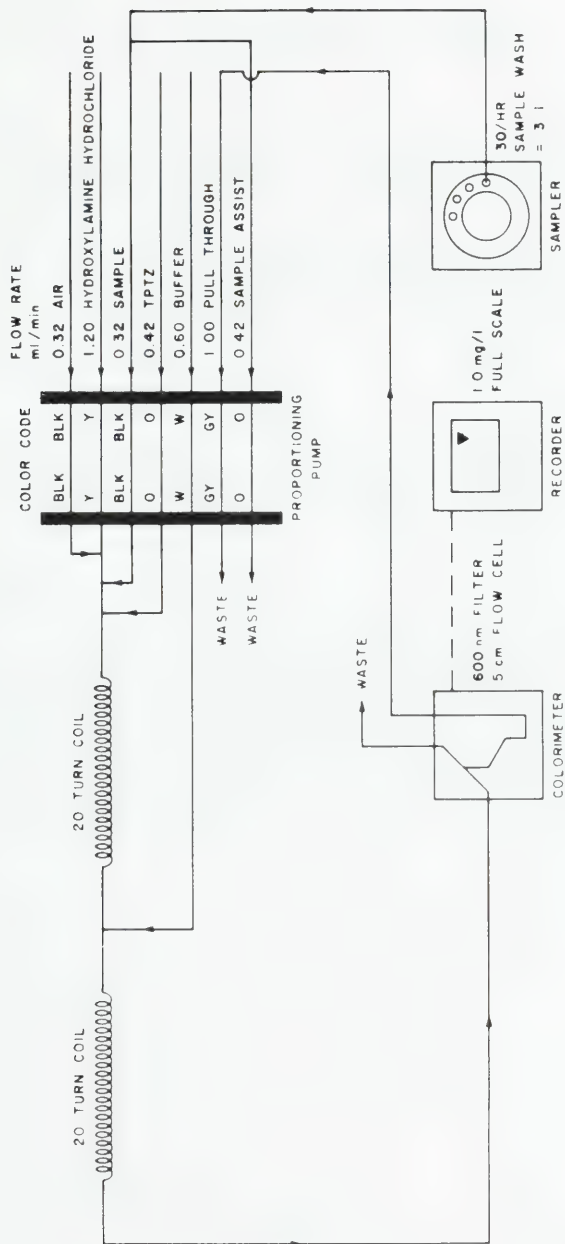


FIGURE 1 — AUTOANALYZER AII SYSTEM FOR TOTAL IRON DETERMINATION IN SURFACE WATERS.

THE DETERMINATION OF MAGNESIUM

Magnesium is abundant and widespread, particularly in the soils and Paleozoic rocks of Southern Ontario where it occurs mainly with calcium, but in smaller quantities. In Ontario waters magnesium concentrations are second only to calcium and generally make up from one-quarter to one-half of the hardness.

Magnesium is essential to both plants and animals. It is non-toxic, but may produce a cathartic effect, particularly in non-acclimatized individuals, at levels greater than 125 mg/l. Concentrations in this range are rarely found in Ontario surface and ground waters.

Sample Handling and Preservation

Water

Glass or polyethylene bottles are acceptable for sample collection. No preservation is required for surface or ground water samples. For industrial waste samples, soil and vegetation analyzed by atomic absorption or ICP see Determination of Trace Metals by Atomic Spectroscopy.

Selection of Method

Four approaches are used for the determination of magnesium in surface and ground waters, industrial waste, soil and vegetation. Method A calculates magnesium values from the difference between the total and calcium hardness results and is usually applied to drinking water samples. It is described only in summary. Method B, automated analyses by atomic absorption spectrophotometry (AAS), is used routinely for surface waters and precipitation samples. A manual atomic absorption method and an ICP method are used for industrial waste, soil, vegetation and other matrices and are described in the Determination of Trace Metals by Atomic Spectroscopy. Manual atomic absorption is also used in the regional laboratories.

MAGNESIUM

Calculation Method A

SUMMARY

Matrix.	This method is used on drinking water samples.
Substance determined.	Mathematical difference between total and calcium hardness reported as mg/l Mg.
Interpretation of results.	The magnesium value is obtained by subtracting a calcium measurement from a hardness measurement using the appropriate conversion factors. If the major hardness producing ions present in the sample are calcium and magnesium then this calculated value closely approximates the actual magnesium content. If however, other hardness producing metallic ions are present (i.e. iron, aluminum, manganese etc.) and are titrated as hardness, then the calculated magnesium will not approximate the actual value in the sample.
Principle of method.	A magnesium value is obtained by calculation: $(\text{Total hardness as CaCO}_3 - \text{calcium hardness as CaCO}_3) \times 0.243 = \text{mg/l magnesium as Mg}^{++}.$ The total hardness is obtained by titration with EDTA (see Determination of Hardness) and the calcium hardness can be obtained by either titration by EDTA (see Determination of Calcium Method A) multiplied by the appropriate factor or by atomic absorption spectrophotometry (see Determination of Calcium Method B) and (Determination of Trace Metals by Atomic Spectroscopy), multiplied by the appropriate factor. The normal method for obtaining the calcium value for this procedure is by EDTA titration.
Time required for analysis.	As many as sixty magnesium results can be calculated, in a batch, in one hour.
Range of application.	Dependent on methods used for hardness and calcium analysis, usually 2 to 300 mg/l. Higher levels can also be calculated.
Standard deviation.	Dependent on methods used for hardness and calcium analysis, usually ± 3 mg/l
Accuracy	Dependent on methods used for hardness and calcium analysis, usually $\pm 1\%$.
Detection criteria.	Dependent on methods used for hardness and calcium analysis, usually 2 mg/l.

Interferences and shortcomings.

For sewage and industrial waste samples the presence of divalent metals, other than calcium and magnesium, can contribute to the measured total hardness, which in turn will affect the concentration of the magnesium result reported.

Minimum volume of sample.

None, other than the amounts required for the total and calcium hardness.

Preservation and sample container.

None, other than what is recommended for total and calcium hardness.

Safety considerations.

See the Determination of Hardness.

MAGNESIUM

Automated Atomic Absorption Method B

SUMMARY

Matrix.	This method is used on surface waters and precipitation samples.
Substance determined.	Magnesium ion Mg^{++} .
Interpretation of results.	Results are reported as mg/l Mg.
Principle of method.	An automated atomic absorption method is used to measure the concentration of magnesium ions. The sample is diluted with a releasing agent, lanthanum chloride, prior to aspiration into the burner flame.
Time required for analysis.	About 200 samples may be analyzed in one day.
Range of application.	Surface waters: a) 0.05 - 5.00 mg/l b) 5.00 - 50.0 mg/l Precipitation: 0.005 - 0.500 mg/l
Standard deviation.	Relative standard deviations are: 2.4% for precipitation samples, 1.5% for low range surface waters and 1.9% for high range surface waters.
Accuracy.	Recoveries for quality control standards are 95% and 96% for surface water and precipitation ranges, respectively.
Detection criteria.	0.007 mg/l for precipitation samples, 0.069 mg/l for low range surface waters and 0.670 mg/l for high range surface waters.
Interferences and shortcomings.	Metals such as sodium, potassium, and calcium cause no interference at concentrations less than 400 mg/l. Aluminum, silicates and carbonates tend to depress the absorbance if present in significant quantities. These interferences are controlled by addition of lanthanum chloride to the sample prior to aspiration.
Minimum volume of sample	50 ml for surface waters, 10 ml for precipitation.

**Preservation and
sample container.**

Glass or polyethylene bottles are acceptable for surface and ground water samples. For industrial waste samples see Determination of Trace Metals by Atomic Spectroscopy.

**Safety
considerations.**

The possibility of burner flash-back or explosion is always present when using flame atomic absorption apparatus. The manufacturer's instructions for burner ignition, use and shut-down should always be rigorously followed, and the waste trap filled with water at all times. Standard safety procedures should be employed when working with compressed gas cylinders. Caution should be exercised during the preparation of the lanthanum chloride since concentrated acid is used.

MAGNESIUM

Automated Atomic Absorption Method B

1. Introduction

The sample under test, automatically mixed with a releasing agent, lanthanum chloride, is aspirated as a fine mist into the air-acetylene flame of an atomic absorption spectrophotometer. Light emitted from a hollow cathode lamp at a characteristic wavelength for magnesium, is directed through the flame into a monochromator and onto a detector. Magnesium atoms, heated in the flame absorb this light and the detector measures the decreased intensity of the resulting beam. The amount of light absorbed is directly proportional to the concentration of magnesium in the sample, and is recorded on a strip-chart recorder as a series of peaks, then compared to a calibration curve derived from simultaneously tested standards. The calibration curve is linear throughout the working range.

2. Interferences and Shortcomings

In the AAS method for magnesium there are few significant interferences present in natural waters. Aluminum, silicates and carbonates, if present in substantial quantities, depress the absorbance of magnesium. These interferences are controlled by the addition of lanthanum chloride to the sample prior to aspiration into the flame.

Partial clogging of the burner nebulizer and consequent reduction in aspiration rates may result from processing samples containing large amounts of suspended solids; pre-filtration of samples is advisable in such cases.

3. Apparatus

- 3.1. Atomic absorption spectrophotometer, a Varian AA275 is used for precipitation samples and a Pye-Unicam SPI 900 is used for surface waters.
- 3.2. Chart recorder.
- 3.3. Sample changer, a Technicon sampler is used for precipitation samples and a Gilson sampler is used for surface waters.
- 3.4. Proportioning pump, pump tubing and assorted manifold glassware as in Figures 1, 2 and 3.
- 3.5. Sample tubes, disposable plastic specimen tubes are used for precipitation samples.
- 3.6. Air and acetylene, suitable for atomic absorption analysis.

NOTE: The burner head should be cleaned frequently with detergent and rinsed thoroughly with distilled water.

4. Reagents

- 4.1. Lanthanum oxide (La_2O_3), reagent grade powder.
- 4.2. Hydrochloric acid, (HCl), concentrated, Aristar grade.
- 4.3. Magnesium metal, reagent grade ribbon, high purity.

Surface Waters

4.4. Magnesium Stock Solution (20,000 mg/l Mg)

In a 500 ml volumetric flask, add 10.0000 g magnesium ribbon (freshly cleaned and dissolved in 1:1 (v/v) hydrochloric acid) to distilled, deionized water and dilute to the mark with distilled, deionized water.

NOTE: For calcium, sodium and potassium stock solutions see individual methods.

4.5. Combined Intermediate Solution (low range 0.05 – 5.00 mg/l)

Pipette 5 ml magnesium stock solution into a 1 liter volumetric flask. If sodium, potassium and calcium are also to be determined transfer 5 ml sodium stock solution, 25 ml potassium stock solution and 10 ml calcium stock solution into the same flask and dilute to the mark with distilled, deionized water. This solution has the following concentrations: 100 mg/l magnesium; 200 mg/l sodium; 100 mg/l potassium and 400 mg/l calcium.

4.6. Combined Intermediate Solution (high range, 5.00 – 50.00 mg/l)

Pipette 50 ml of each of the magnesium, sodium, potassium and calcium stock solutions into the same volumetric flask and dilute to 1 liter with distilled, deionized water. This gives the following concentrations: 1000 mg/l magnesium; 2000 mg/l sodium; 200 mg/l potassium and 2000 mg/l calcium.

4.7. Combined Calibration Standards (0.05 – 5.00 mg/l range)

In volumetric flasks, dilute the following aliquots of low range intermediate solution (reagent 4.5) to 1 liter with distilled, deionized water.

Final Concentration mg/l

Aliquot	Magnesium	Sodium	Potassium	Calcium
50 ml	5	10	5	20
40 ml	4	8	4	16
30 ml	3	6	3	12
20 ml	2	4	2	8
10 ml	1	2	1	4
5 ml	0.5	1	0.5	2

4.8. Combined Calibration Standards (5.00 – 50.00 mg/l range)

In volumetric flasks, dilute the following aliquots of high range intermediate solution (reagent 4.6) to 1 liter with distilled, deionized water.

Final Concentration mg/l

Aliquot	Magnesium	Sodium	Potassium	Calcium
50	50	100	10	100
40	40	80	8	80
30	30	60	6	60
20	20	40	4	40
10	10	20	2	20
5	5	10	1	10

4.9. Quality Control Stock Solution (for low range; 72 mg/l Mg)

In a 1 liter volumetric flask add 0.0720 g magnesium ribbon (freshly cleaned and dissolved in 1:1 (v/v) hydrochloric acid) to distilled, deionized water and dilute to the mark with distilled, deionized water. Use a different batch of magnesium metal than that used for reagent 4.3.

NOTE: If sodium, potassium and calcium are also to be determined, prepare separate stock solution as outlined in the Determination of Sodium, the Determination of Potassium and the Determination of Calcium.

4.10. Quality Control Working Standards (low range, 0.05 - 5.00 mg/l)

QC-A: Dilute 50 ml of quality control stock solution (reagent 4.9) to 1 liter in a volumetric flask with distilled, deionized water. This solution is 72% of scale.

QC-B: Dilute 20 ml of quality control stock solution (reagent 4.9) to 1 liter in a volumetric flask with distilled, deionized water. This solution is 28.8% of scale.

4.11. Quality Control Stock Solution (for high range; 720 mg/l)

In a 1 liter volumetric flask add 0.7200 g magnesium ribbon (freshly cleaned and dissolved in 1:1 (v/v) hydrochloric acid) to distilled, deionized water and dilute to the mark. Use a different batch of magnesium metal than the one used for preparation of reagent 4.3.

NOTE: If sodium, potassium and calcium are also to be determined prepare a separate stock as outlined in the Determination of Sodium, the Determination of Potassium and the Determination of Calcium.

4.12. Quality Control Working Standards (high range 5.00 - 50.00 mg/l)

QC-A: Dilute 50 ml quality control stock solution (reagent 4.11) to 1 liter in a volumetric flask with distilled, deionized water. This solution is 72% of scale.

QC-B: Dilute 20 ml quality control stock solution (reagent 4.11) to 1 liter in a volumetric flask with distilled, deionized water. This solution is 28.8% of scale.

NOTE: Prepare sufficient solution for at least 20 days of analysis. Prepare new QC-A's and QC-B's early and monitor their response for at least 3 days prior to adopting them.

4.13. Lanthanum Chloride Solution (Surface Waters)

Dissolve 16.00 g lanthanum oxide in 200 ml distilled, deionized water containing 30 ml concentrated hydrochloric acid. When the salt is completely dissolved, dilute to 4000 ml with distilled, deionized water and mix thoroughly. The pH of this solution should be approximately 1.7. Store solution in a plastic bottle.

Precipitation Samples

4.14. Magnesium Stock Solution (1,000 mg/l Mg)

In a 1000 ml volumetric flask add 1.0000 g magnesium ribbon (freshly cleaned and dissolved in 1:10 (v/v) hydrochloric acid) to distilled, deionized water and dilute to the mark with distilled, deionized water.

NOTE: If sodium, potassium and calcium and also to be determined prepare separate stock solutions according to the methods described in the Determination of Sodium, the Determination of Potassium and the Determination of Calcium.

4.15. Combined Calcium-Magnesium Intermediate Solution

In a 1 liter volumetric flask, dilute 10 ml calcium stock solution and 10 ml magnesium stock solution to the mark with distilled, deionized water. This gives a calcium concentration of 40 mg/l and a magnesium concentration of 10 mg/l.

NOTE: For the measurement of sodium and potassium a combined intermediate solution is also prepared by diluting 20 ml sodium stock solution and 20 ml potassium stock solution to 1 liter with distilled, deionized water. This gives a sodium and potassium concentration of 20 mg/l. See the Determination of Sodium and the Determination of Potassium.

4.16. Combined Calcium and Magnesium Working Standards

Into volumetric flasks, pipette 5.00, 10.00, 20.00, 30.00, 40.00 and 50.00 ml aliquots of the combined intermediate solution (reagent 4.15) to 1 liter with distilled, deionized water. This gives working standards with calcium concentrations of 0.20, 0.40, 0.80, 1.20, 1.60 and 2.00 mg/l respectively and magnesium concentrations of 0.05, 0.10, 0.20, 0.30, 0.40 and 0.50 mg/l respectively.

NOTE: Similar sodium and potassium working standards are prepared by diluting aliquots of combined sodium-potassium intermediate solution to 1 liter in volumetric flask to give a set of standards with concentrations of 0.10, 0.20, 0.40, 0.60, 0.80, 1.00 mg/l sodium respectively and 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00 mg/l potassium respectively.

4.17. Magnesium Quality Control Stock Solution (1000 mg/l Mg)

Prepare a magnesium quality control solution which is identical to reagent 4.14 but using a different batch of magnesium ribbon.

NOTE: For calcium, sodium and potassium quality control stock solutions see the Determination of Calcium, the Determination of Sodium and the Determination of Potassium.

4.18. Quality Control Intermediate Solution

Pipette 10 ml calcium quality control stock (see Determination of Calcium) and 10 ml magnesium quality control stock solution reagent 4.17) into a 1 liter

volumetric flask. This gives a calcium concentration of 40 mg/l and a magnesium concentration of 10 mg/l.

NOTE: If sodium and potassium quality control intermediate solutions are also required, prepare a combined solution with 20 mg/l sodium and 20 mg/l potassium using the stock solutions for surface waters outlined in the Determination of Sodium and the Determination of Potassium.

4.19. Quality Control Working Solutions

QC-A: In a volumetric flask, dilute 30.0 ml quality control intermediate solution with distilled, deionized water to 1 liter. This solution has a calcium concentration of 1.20 mg/l and a magnesium concentration of 0.30 mg/l.

QC-B: In a volumetric flask, dilute 5.0 ml quality control intermediate solution with distilled, deionized water to 1 liter. This solution has a calcium concentration of 0.20 mg/l and a magnesium concentration of 0.05 mg/l.

NOTE: If sodium and potassium quality control working solutions are also required prepare solutions with a sodium concentration of 0.60 mg/l and a potassium concentration of 0.60 mg/l for the QC-A and sodium concentration of 0.10 mg/l and a potassium concentration of 0.10 mg/l for the QC-B. See individual methods for a detailed description.

Prepare QC-A's and -B's for at least 20 days of analysis. Prepare new QC-A's and QC-B's early and monitor their response for at least 3 days prior to adopting them.

4.20. Lanthanum Chloride Solution (Precipitation Samples)

Dissolve 8 g lanthanum oxide in 200 ml distilled, deionized water containing 20 ml concentrated hydrochloric acid. When salt is completely dissolved dilute to 1 liter with distilled, deionized water.

NOTE: Lanthanum chloride solution is proportioned with sample so that the approximate lanthanum concentration at the burner head is 680 mg/l.

5. Procedure

5.1. Surface Waters

ANALYSES ARE PERFORMED USING AN AIR-ACETYLENE FLAME IN AN IDENTICAL MANNER AS CALCIUM (SEE THE DETERMINATION OF CALCIUM, METHOD B) WITH THE FOLLOWING EXCEPTION

5.1.1. Wavelength 285.2 nm (magnesium lamp)

NOTE: The danger of burner flash-back and/or explosion is always present while using flame atomic absorption apparatus. The manufacturer's procedures should be carefully followed for ignition, use, and shut-down of the burner. Normal safety precautions must be exercised when transporting and using compressed gas cylinders.

THE AAS UNIT MUST NOT BE LEFT UNATTENDED WHILE IT IS IN OPERATION.

For all other details of the AAS operation refer to the Determination of Calcium.

5.2. Precipitation Samples

THE PROCEDURE FOR MAGNESIUM DETERMINATION IS IDENTICAL TO CALCIUM ANALYSIS (SEE THE DETERMINATION OF CALCIUM) WITH THE FOLLOWING EXCEPTIONS:

- 5.2.1. wavelength = 285.2 nm
burner height = optimum level for the element

For all other details of the AAS operation, the reader is referred to the Determination of Calcium. See also safety note in 5.1.

6. Calculation and Reporting

Sample absorbance is compared to the absorbance of known standards and magnesium concentration is read from a calibration curve. Results are multiplied by a dilution factor, if necessary.

Results are reported as follows:

Surface Water	Report
0.05 - 5.00	to nearest 0.05
5.00 - 50.0	to nearest 0.5
Precipitation	Report
<0.005	as <0.005
0.005 - 0.500	to nearest 0.005

7. Precision and Accuracy

Standard deviations based on within-run duplicate samples are as follows:

Sample Type	Range (mg/l)	S _{ld}	S _{md}	S _{hd}
Surface waters	0.05 - 5.00	0.042	0.044	0.055
	5.00 - 50.00	0.407	0.333	
Precipitation	0.005 - 0.500	0.004	0.020	0.036 *

* Data available only for old range 0.01 - 1.00.

Where:

S_{ld} = standard deviation for 0 - 20% of range

S_{md} = standard deviation for 20 - 50% of range

S_{hd} = standard deviation for 50 - 100% of range

Recoveries for quality control standards are 95% and 96% for surface water and precipitation ranges respectively.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater, 13th edition, APHA, Washington, D.C., 316.
- 8.2. Lindow, O. (1979). Determination of trace levels of calcium, magnesium, sodium and potassium by atomic absorption spectrophotometry-precipitation samples. Ministry of the Environment, Laboratory Services Branch, Rexdale, Ontario.
- 8.3. United States Environmental Protection Agency (1974). Methods of Chemical Analysis of Water and Wastes. U.S. EPA, Washington, D.C. 147.

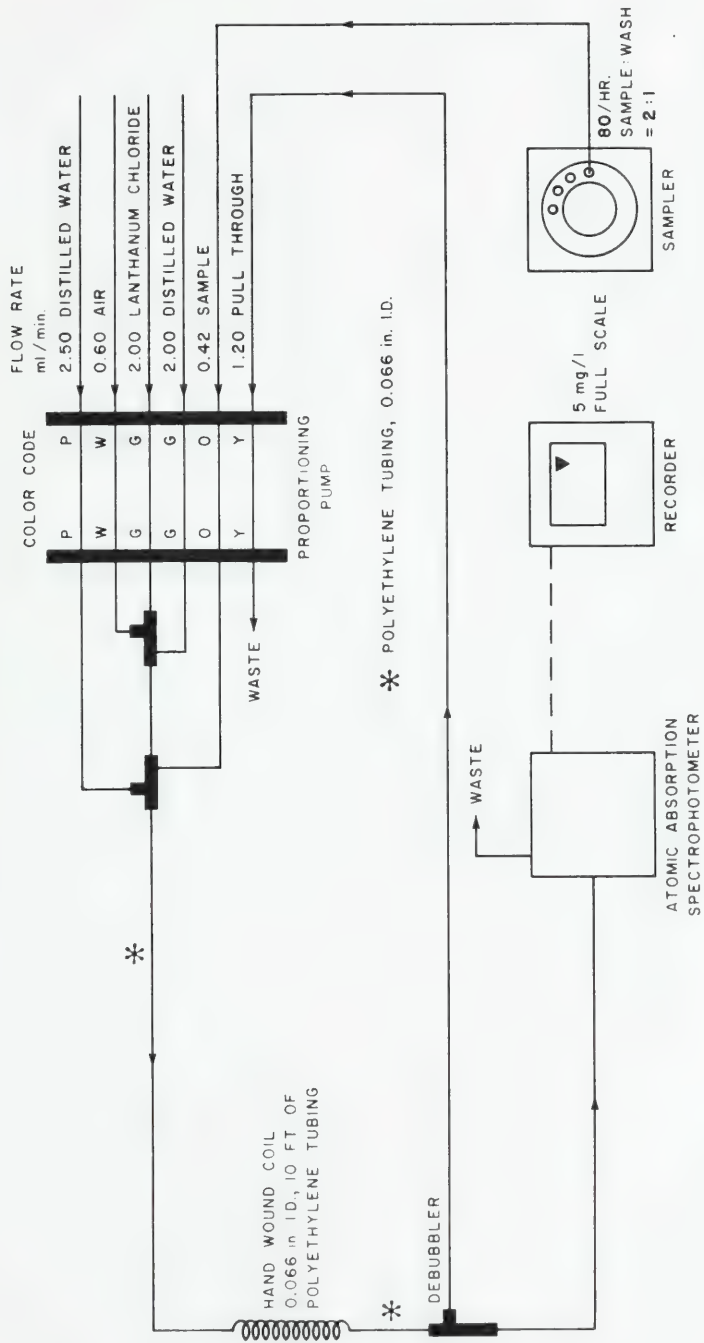


FIGURE 1 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR LOW LEVEL MAGNESIUM DETERMINATIONS IN SURFACE WATERS

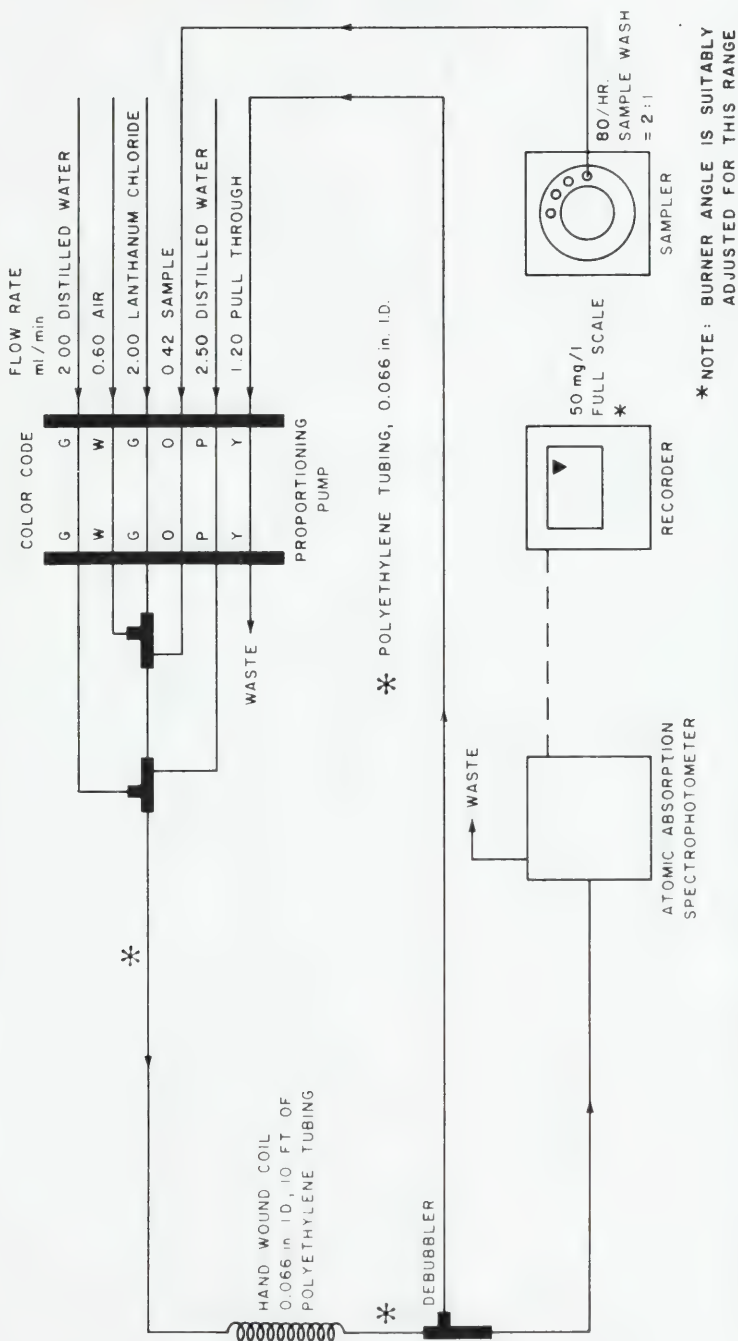


FIGURE 2 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR HIGH LEVEL MAGNESIUM DETERMINATIONS IN SURFACE WATERS

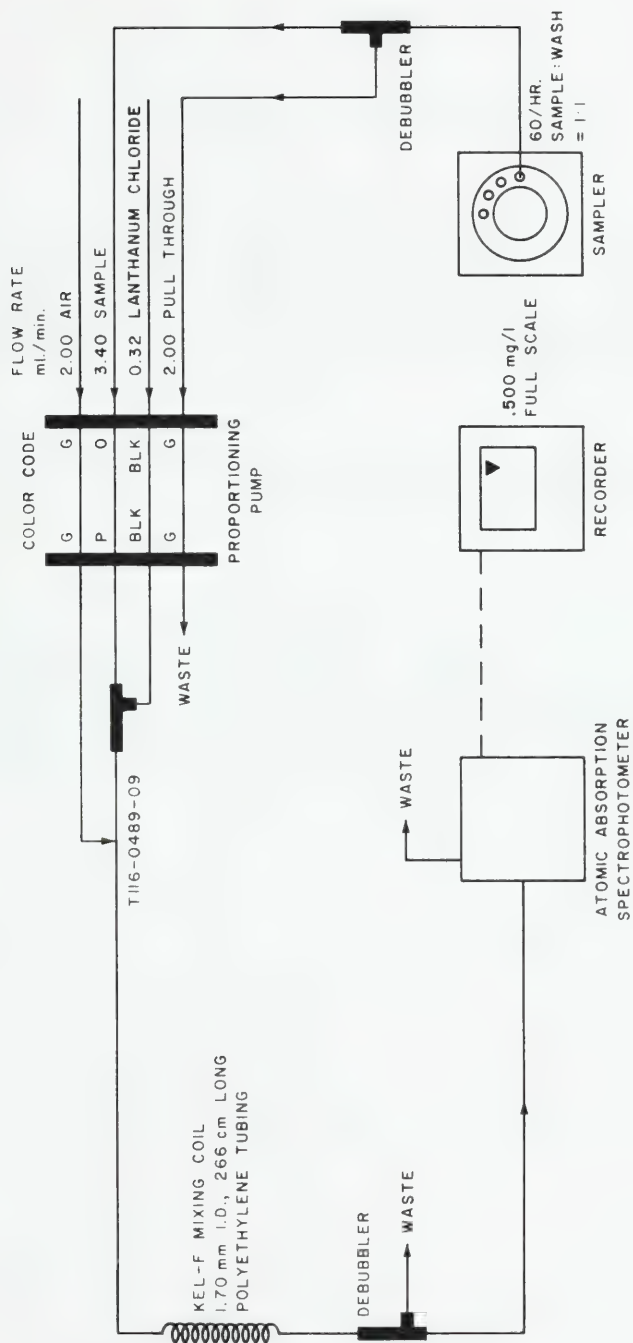


FIGURE 3 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR MAGNESIUM DETERMINATIONS ON PRECIPITATION SAMPLES

THE DETERMINATION OF MANGANESE

Manganese is the second most abundant heavy metal in the earth's crust (0.085%), and is invariably present in natural waters but at concentrations which rarely exceed 1 mg/l. Manganese is a required nutritional element for man and is normally not considered toxic, but prolonged exposure can result in impaired motor facilities. Excessive concentrations are undesirable in domestic water supplies as manganese imparts tenacious stains to laundry and plumbing fixtures. For these reasons, the maximum permissible criteria for domestic water supplies in Ontario has been established as 0.05 mg/l manganese.

Sample Handling and Preservation

Glass or plastic bottles are used for sample collection. Manganese precipitation and loss by adsorption onto the walls of the container, is prevented by the addition of 1 ml concentrated nitric acid per liter.

Selection of Method

Three methods are employed for the determination of manganese. Method A is an automated colorimetric method employing the complexing agent formaldoxime and is used for routine analysis of natural waters of low concentrations. The other methods involve Atomic Spectroscopic analysis as described in the Determination of Trace Metals. The colorimetric procedure is used when iron and manganese are being analyzed in conjunction with routine water quality parameters excluding trace metals. Iron is analyzed simultaneously. For details, see the Determination of Iron.

TOTAL MANGANESE
Automated Colorimetry Method A

SUMMARY

Matrix.	This method is used routinely on river and lake samples.
Substance determined.	Total manganese as Mn. This method does not differentiate among the different oxidation states.
Interpretation of results.	Results are reported as mg/l Mn. Most rivers and lakes contain 0 - 0.05 mg/l manganese, but the concentration can exceed 1 mg/l during the spring runoff due to the high portion of particulate matter.
Principle of method.	An aliquot of sample is acidified with a sulphuric acid-hydroxyl-ammonium chloride solution and autoclaved for one hour at approximately 121°C to solubilize the manganese content. Manganese is reacted with formaldoxime at pH 10 to form a reddish-brown complex. This color, which is proportional to manganese present, is measured at 480 nm.
Time required for analysis.	Two hours are required for a single analysis, but 150 samples can be analyzed per day.
Range of application.	Normal working range is 0.002 to 0.200 mg/l. Higher levels are determined by dilution.
Standard deviation.	± 0.0012 for 0 - 20% of working range; 0.0021 for 20 - 50% of range; ± 0.0029 for 50 - 100% of range, based on within-run duplicate analyses.
Accuracy.	Recoveries of two quality control standards are 98% and 98.6% respectively. The average recovery on two standards taken through the digestion process is 100.1%.
Detection criteria.	0.002 mg/l Mn.
Interferences and shortcomings.	Excessive sample color, cobaltous and nickel ions in excess of 1 and 2 mg/l respectively and sulphide in excess of 0.5 mg/l may interfere.

**Minimum volume
of sample.** 50 ml.

**Preservation and
sample container.** Glass or plastic bottles are acceptable for sample collection. If manganese is to be analyzed as part of a general Trace Metal Scan, samples should be collected in plastic containers and should be preserved with 1 ml of concentrated nitric acid per liter of sample. See the Determination of Trace Metals.

**Safety
considerations.** Normal laboratory safety should be observed.

TOTAL MANGANESE

Automated Colorimetry Method A

1. Introduction

Manganese is solubilized by digesting an aliquot with a sulphuric acid-hydroxylammonium chloride reagent in an autoclave at approximately 121°C for one hour. Using a Technicon AAII AutoAnalyzer system the manganese content of the sample is determined colorimetrically by formation of its formaldoxime complex in a reducing media at pH 10. The pH of the system is controlled by an ammonia-ammonium chloride buffer, and complexing agents are utilized to suppress cation interference. A reference stream is operated parallel to the color producing stream to minimize the effects of sample color and iron content. The reference stream includes the sample and all the reagents at the same concentrations as found in the color stream, but the order of reagent addition is altered to prevent the formation of the manganese-formaldoxime complex.

2. Interferences and Shortcomings

Positive interferences due to cations are adequately controlled by the complexing agents: ascorbic acid, EDTA, and hydroxylammonium chloride. The system will tolerate 1 mg/l Co^{2+} , 2 mg/l Ni^{2+} , and at least 5 mg/l of the other heavy metals tested including Fe^{3+} . Sulphide causes a 5% negative interference at the 1 mg/l level and tannic and humic acids cause a 4% positive interference at the 5 and 10 mg/l levels respectively.

3. Apparatus

ALL GLASSWARE MUST BE RINSED WITH DILUTE HYDROCHLORIC ACID PRIOR TO USE.

3.1. AutoAnalyzer II channel (Technicon) consisting of:

Sampler (large industrial model)

Proportioning pump III including assorted glassware and pump tubing (see Figure 1). The only metal allowed to come in contact with the sample is platinum-iridium (such as those imbedded in injection fittings) due to the acidic nature of the digested sample.

Colorimeter equipped with voltage stabilizer, 480 nm filters and 50mm x 1.5 mm flowcells.

3.2. Chart recorder (capable of 10 mV full scale input).

3.3. Culture tubes, Pyrex glass, (25mm x 150 mm) equipped with Nalgene size 24 polypropylene screw caps (approximately 200).

- 3.4. Autoclave equipped with an automatic slow exhaust and capable of being operated at 121°C.
- 3.5. Oxford pipettor.
- 3.6. Assorted glassware: volumetric flasks, pipettes, etc.

4. Reagents

- 4.1. Standard manganese solution, (1000 mg/l), BDH prepared.
- 4.2. Hydrochloric acid (HCl), concentrated, reagent grade.
- 4.3. Sulphuric acid (H₂SO₄), concentrated, reagent grade.
- 4.4. Hydroxylammonium chloride (NH₂OH.HCl), reagent grade powder.
- 4.5. Formaldehyde, (HCHO), 37% reagent grade.
- 4.6. Ammonium chloride, (NH₄Cl), reagent grade, crystal.
- 4.7. Ammonium hydroxide, (NH₄OH), concentrated, reagent grade.
- 4.8. Ethylenediaminetetraacetic acid (EDTA) disodium salt, reagent grade crystal.
- 4.9. L-ascorbic acid, (C₆H₈O₆), reagent grade powder.
- 4.10. Potassium ferricyanide (K₃Fe(CN)₆), reagent grade crystals.
- 4.11. Standard iron solution (1000 mg/l) BDH prepared.

4.12. Digestion Acid

Dissolve 12.08 g hydroxylammonium chloride in approximately 100 ml distilled, deionized water. While mixing, carefully add 800 ml of concentrated sulphuric acid and dilute to 4 liters.

NOTE: Wear eye and hand protection.

4.13. Acidified Blank Solution

Prepare a blank solution by diluting 62 ml digestion acid to 1 liter with distilled, deionized water.

4.14. Formaldoxime Solution

Dissolve 250 g hydroxylammonium chloride in 1300 ml distilled, deionized water. Add 100 ml 37% formaldehyde and dilute to 2 liters in a volumetric flask.

4.15. EDTA (disodium salt) Solution

Dissolve 37.2 g EDTA and 100 g hydroxylammonium chloride in distilled, deionized water. Dilute to 1 liter.

4.16. L-ascorbic acid

Dissolve 21.0 g L-ascorbic acid in distilled, deionized water and dilute to 250 ml in a volumetric flask.

4.17. Ammonia Buffer

Dissolve 100 g ammonium chloride in 375 ml concentrated ammonium hydroxide contained in a Pyrex reagent bottle.

NOTE: Due to the volatile nature of ammonia, preparation must be done in a fume hood. Wear eye and hand protection.

4.18. Manganese Stock Solution (4 mg/l Mn)

Pipette 4.0 ml BDH standard manganese solution (reagent 4.1) into a 1 liter volumetric flask. Add 1 ml concentrated sulphuric acid and dilute to volume with distilled, deionized water. This solution is stable for at least six months.

4.19. Manganese Working Standards

Dilute 10 ml and 40 ml aliquots of the manganese stock solution to prepared in 4.18 1 liter with distilled, deionized water containing 1 ml concentrated sulphuric acid. This gives 2 standards with manganese concentrations of 0.04 and 0.16 mg/l respectively.

4.20. Quality Control Stock Solution

Use a different batch of BDH prepared manganese standard solution (1000 mg/l) than the one used for preparation of reagent 4.18.

4.21. Quality Control Standard Solution (16 mg/l Mn)

Dilute 8 ml of the quality control stock solution prepared in 4.20 to 500 ml with distilled, deionized water containing 1 ml concentrated sulphuric acid.

4.22. Quality Control Working Solutions

QC-A: Dilute 30 ml of the quality control standard solution prepared in 4.21 to 4 liters with distilled, deionized water containing 4 ml concentrated sulphuric acid. This solution has a manganese concentration of 0.12 mg/l.

QC-B: Dilute 20 ml of the quality control standard solution prepared in 4.21 to 4 liters with distilled, deionized water containing 4 ml concentrated sulphuric acid. This solution has a manganese concentration of 0.08 mg/l

Long Term Blank: Add 4 ml concentrated sulphuric acid to 4 liters of distilled, deionized water.

4.23. Sensitivity Checks

High (80% of scale): To 40 ml of manganese stock solution from 4.18, add 62 ml digestion acid and dilute to 1 liter with distilled, deionized water.

Low (20% of scale): To 10 ml of manganese stock solution from 4.18, add 62 ml digestion acid and dilute to 1 liter with distilled, deionized water.

4.24. Iron Recovery Stock Solution (1000 mg/l Fe)

Dissolve 5.896 g potassium ferricyanide in distilled, deionized water and dilute to 1 liter.

4.25. Combined Iron and Manganese Recovery Solution

Dilute 20 ml of the iron recovery stock solution (4.24) and 4 ml of the manganese standard solution (4.1) to 1 liter with distilled, deionized water. This gives a solution with 20 mg/l iron and 4 mg/l manganese.

4.26. Digested Standards

Dilute each of 10 and 40 ml aliquots of the combined iron and manganese recovery solution to 1 liter with distilled, deionized water. This gives digested standards of 0.04 and 0.16 mg/l manganese. These solutions are analyzed in duplicate and quadruplicate respectively, per run.

4.27. Iron Interference Check (5 mg/l Fe)

Dilute 5 ml standard iron solution (reagent 4.11) to 1 liter with distilled, deionized water containing 1 ml concentrated sulphuric acid.

4.28. Wash Water (1.25% v/v)

Add 50 ml concentrated sulphuric acid to 4 liters distilled, deionized water.

4.29 Hydrochloric Acid (4% v/v)

Add 160 ml concentrated hydrochloric acid to 3500 ml distilled, deionized water slowly with stirring. Dilute to 4 liters.

NOTE: Wear eye and hand protection.

5. Procedure

REFER TO MANUFACTURER'S MANUAL FOR AUTOANALYZER OPERATION AND CLEANING PROCEDURES.

NOTE: Digestion of from 100 - 200 samples together allows for the most efficient use of time.

- 5.1. Clean the culture tubes and screw caps between analyses by soaking them in a 4% HCl solution. Rinse with distilled water and dry.
- 5.2. Pipette 30.0 ml of each calibration standard, digested standards, iron interference check, quality control solutions, long term blank, daily blank and sample into cleaned culture tubes.
- 5.3. Add 2 ml digestion acid to all samples and standards.
- 5.4. Screw polypropylene lined caps on tightly, mix thoroughly and place tubes in racks on an enamel tray. Autoclave for one hour at 121°C using slow exhaust setting. When pressure has been discharged, set the autoclave door ajar. After 10 minutes open the door, remove the tubes, and allow them to cool to room temperature. Mix to remove condensate from the walls of the tubes and remove caps.
- 5.5. Set up AutoAnalyzer ensuring that buffer is present in the manifold and that 10% sulphuric acid traps are connected to the air inlets of the ammonia buffer and waste reagent bottles.

NOTE: The synchronization of the A and B sides of the colorimeter may be carried out using methyl red indicator as a sample, and with the reagent lines placed in the acidified wash water. Allow an air separation to go into each sample line then aspirate the methyl red for about 15 seconds. Replace the sample probe in wash water and observe the dye fronts as they pass the tube which leads to the light path on the flowcell. Synchronization is achieved by shortening the sample pump tube of the side which is lagging. (Roughly the same length as the difference between the two fronts.) Repeat the procedure until the two fronts are matched.

5.6. Each run includes the following:

0.16 mg/l standard (HIGH); standards 0.04 and 0.16 mg Mn/l (STDS); digestion acid blank (DBL); long term blank (LTBL); acidified blank solution (BL); QC-A (A); QC-B (B); iron interference check (Fe); digested recovery standards (REC); sensitivity monitoring standards high (H) and low (L); and samples in groups of 10 or less (10).

5.7. Samples are loaded into the drum in the following order:

HIGH; BL; HIGH; BL; STDS; BL; LTBL; A; B; BL; Fe; BL; 2 DBL; REC; BL; L; H; BL; n (10, BL, 10, L, H, BL).

Where n = number of repetitive units of samples.

Multiple working standards are used only to establish linearity and a calibration strip. For day to day calibration, the 0.040 and 0.160 mg Mn/l standards are recommended for instrument calibration.

5.8. Commence run whenever there is sufficient work to allow uninterrupted operation of the AutoAnalyzer.

5.9. The second HIGH is adjusted to read 80% of scale on the recorder by use of the Std. Cal. control (after daily operation has been established, this adjustment should be minimal). Use the calibration strip (prepared from a complete set of analyzed standards) and read the values for the quality control samples, long term blank, iron interference solution, digested acid blank and recovery standards. If these values agree with previous results (or theoretical), let the run continue. If they do not agree, the run must be stopped and the problem corrected; then restarted from the beginning.

5.10. Read each sample peak using the calibration strip and record the result for the corresponding sample number.

6. Calculation and Reporting

Multiply the reading by the dilution factor

$$DF = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result for the appropriate sample number. A minimum of three significant figures are shown when reporting to three decimal places with a minimum reporting value of less than 0.002 mg/l Mn.

7. Precision and Accuracy

The precision is determined by analyzing samples in duplicate.

Standard deviations are ± 0.0012 for the 0.002 - 0.04 mg/l concentration range; ± 0.0021 for the 0.04 - 0.1 mg/l range and ± 0.0029 for the 0.1 - 0.2 mg/l range.

Recoveries of two quality control standards are 98% and 98.6% respectively. The average recovery on two standards taken through the digestion process is 100.1%

8. Bibliography

- 8.1. Abdullah, M.J. (1968). The automatic determination of manganese in silicate rocks and sediment. *Analytica Chimica Acta.* **40**: 526 - 530.
- 8.2. Cheeseman, R.V. and Wilson, A.L. (1972). A method for the determination of manganese in water. *Water Research Association Paper* 85. 49p.
- 8.3. Henriksen, A. (1966). An automatic modified formaldoxime method for determining low concentrations of manganese in water containing iron. *The Analyst* **9**: 647 - 651.
- 8.4. Morgan, J.J. and Stumm, W. (1965). Analytical chemistry of aqueous manganese. *Journal of the American Water Works Association*, **57**: 107 -119.
- 8.5. Crowther, J. (1978). Semi-automated Procedure for the Determination of Low Levels of Total Manganese. *Analytical Chemistry* **50**: 1041-1043.

THE DETERMINATION OF MERCURY

Geologically, mercury is a rare element and comprises less than 3×10^{-6} per cent of the earth's crust. There are comparatively few locations on the earth's surface where mercury is present in more than trace amounts. Most of the naturally occurring mercury is in the form of highly insoluble mercuric sulfide, the principal component of cinnabar.

Industrial uses of mercury are quite widespread, ranging from small scale uses such as thermometers and electrical switches to the large amounts used in the manufacture of caustic soda and chlorine in the chlor-alkali industry. Mercury compounds were used quite extensively as biocides, fungicides, and in the manufacture of pharmaceuticals and paints.

The recent concern with mercury pollution began with the Minamata case in which hundreds of Japanese were poisoned, resulting in at least fifty fatalities. This was followed by a similar outbreak of poisoning in Niigata, Japan, and in the mid-sixties by reports from Sweden of high levels of mercury in fish and wildlife. As a result of these studies, it was shown that any form of mercury entering an aquatic system could be converted (by micro-organisms and biochemically derived alkylating systems) to methylmercury derivatives. These compounds follow a process of concentration as progressively larger animals feed on smaller ones, until the concentration endangers higher forms of life. Due to the low concentration of mercury that can cause problems and to the various matrix effects which have to be considered, the analytical work associated with this problem is quite complex.

Sample Handling and Preservation

Water

Samples should be taken in 1 liter glass containers and should not be allowed to come into contact with any metal objects. It has been demonstrated that losses of mercury occur when unpreserved samples are stored in polyethylene or glass containers. These losses appear to result from inorganic mercury adsorption on the walls of the container.

For total mercury analysis, the samples should be preserved with potassium dichromate and nitric acid immediately after sampling. The pH must be below 1 and enough 5% potassium dichromate must be added to maintain a pale yellow colour.

Storage of the sample for more than 7 days can result in a considerable loss of mercury even with preservation, therefore analysis of the samples should be carried out as soon as possible after sampling.

A single analysis of water for its methylmercury content requires 4 liters. The samples are submitted in glass bottles with 2 ml of nitric acid per liter of sample added as a preservative. If possible, the samples should be protected from light to prevent the photodecomposition of methylmercury. Special arrangements with laboratory staff must be made prior to the submission of water samples for methylmercury analysis.

Soil and Sediment

Care should be taken when sampling to ensure that samples are representative of the area being examined. Sediment samples should be transported directly to the laboratory in glass jars with nonmetallic caps. Sediments from different sampling points should be kept separate to avoid cross-contamination. Sampling devices and containers must be free of mercury contamination and should be designed so as to minimize the chances of mercury loss by amalgamation. Sediments are not dried.

Soil samples are normally collected in conjunction with vegetation samples as an aid to the differentiation between current and past emission situations. Occasionally, soil samples are collected to establish background conditions.

Soil is collected with a 2 cm (3/4") diameter stainless steel tube. A minimum of 10 cores are taken from the sampling site. All soil samples are collected in triplicate (i.e. minimum 3 x 10 cores) and the collection form is completed comprehensively describing the texture of the soil and the overall sampling site. Each core must be separated into fractional depths of 0 - 5 cm, 5 - 10 cm and 10 - 15 cm, and each level is placed in an appropriately labelled plastic bag or glass jar with a nonmetallic cap for shipping. Upon arrival at laboratory, samples are generally air dried.

Ideally, soils should be sampled from an entirely undisturbed or sodded area and contaminated situations should be matched as closely as possible with conditions existing immediately outside the area.

Vegetation

To ensure correct interpretation of analytical data, the plant species, the age or maturity of leaf tissues, the age of tree or shrub, and the position of sample on the tree or shrub should be recorded for each sample. Usually, foliage is collected from the side of the tree or shrub facing the presumed source of air pollution but, occasionally, a second sample may be taken from the side opposite to the source. Samples are taken by trimming outside growth from ground level up to 7 meters or more and collecting all leaves to provide a composite sample of 500 to 1,000 grams of fresh material.

Current practice dictates the collection of 3 samples from each sampling location (triplicate sampling). Samples are placed in perforated polyethylene bags and transferred to refrigerated storage as soon as possible for processing by the Phytotoxicology Laboratory. Forage samples (grass) are collected by cutting the terminal 25 cm (10") of stems and blades over the representative area to be sampled, at 10 step intervals. Dried flower heads and stalks are discarded and no root material whatsoever is included. The different forage species included in the sample are identified and are representative of the population of the species in the field.

Any sample contaminated by roadside dust should be noted in the accompanying request form. All vegetation samples as collected, are potentially unstable, and will decompose unless properly handled. Vegetation samples can be preserved for a few weeks under refrigeration; when dried at 80°C for 30 hours in a forced draft oven, they become almost permanently stable.

Biological Material (Fish)

Subsequent to weighing, the fish is placed on a wooden board covered with paper towelling. An incision is made with a stainless steel knife on the dorsal surface posterior to the nape, and the body is cut ventrally to a point below and posterior to the pectoral fin. The sex of the fish is noted and recorded.

The fish is cut posteriorly along the dorsal surface. The epaxial musculature (muscle above the lateral line) is stripped from the skin and about 100 g is covered with labelled aluminum foil and frozen immediately to retain moisture and retard protein breakdown.

Selection of Method

A variety of methods are employed for mercury determination depending on the sample matrix and the fraction of mercury under consideration. Method A, measures total mercury in water samples and requires a preliminary digestion procedure followed by mercury determination using flameless atomic absorption spectrophotometry (FAAS). The FAAS technique is rapid, reproducible and relatively devoid of interferences. Method B, is also an FAAS technique however this method is automated. Soil, sediment, vegetation biological materials and sewage sludge are analyzed for total mercury by this method, after a preliminary acid extraction. Method C is a gas chromatographic technique used for methylmercury determinations on water and fish samples. This method requires a lengthy extraction prior to G.C. analysis but is relatively free of interferences.

TOTAL MERCURY

December
1983

Acid Digestion - Manual Flameless Atomic Absorption Method A

SUMMARY

Matrix.	This method is applied routinely to water samples. Sewage and industrial effluent containing low levels of mercury may also be analyzed by this method.
Substance determined.	Total mercury, Hg.
Interpretation of results.	Results are reported as $\mu\text{g/l}$ mercury. Results indicate the level of total mercury in the sample.
Principle of method.	Mercury in the sample is converted to the inorganic form by a digestion procedure. The inorganic mercury is reduced in aqueous solution with stannous chloride. A stream of air is bubbled through the vessel containing the reduced mercury. The air stream carries the mercury vapor into a flow-through absorption cell positioned between a 253.7 nm wavelength light source and a detector. The amount of absorption is proportional to the concentration of mercury in the samples.
Time required for analysis.	A single analysis takes about 4 hours. However, 20 analyses per day can be completed when the samples are tested in batches.
Range of application.	0.05 $\mu\text{g/l}$ to 10 $\mu\text{g/l}$. The range can be extended by dilution.
Standard deviation.	0.03 $\mu\text{g/l}$ on a sample containing 0.2 $\mu\text{g/l}$. 0.14 $\mu\text{g/l}$ on a sample containing 8.8 $\mu\text{g/l}$. (Part of an EPA method evaluation.)
Accuracy.	The average recovery for a series of samples ranging from 0.2 to 10 $\mu\text{g/l}$ was 99.3%.
Detection criteria.	0.05 mg/l . The detection limit is based on the blank value for the particular series of samples being analyzed. In this test, twice the value of the blank is considered the detection limit.

Interferences and shortcomings.

Water vapor can condense on the windows of the absorption cell, causing absorption of radiation at the 253.7 nm resonance line of mercury. However, two systems can be used to remove the water vapor interference: a) a bubbler immersed in an ice bath will condense moisture passing through the system, resulting in a low constant water vapor level and b) a moisture trap containing calcium sulphate will remove moisture from the system. Hydrogen sulphide can interfere, but is eliminated by potassium permanganate. Elevated chloride concentrations are removed by the sulphuric, nitric acid and permanganate oxidation step.

Minimum volume of sample.

At least 250 ml are required if duplicate analyses are to be performed.

Preservation and sample container.

For total mercury, Pyrex glass bottles should be used and the bottle caps should be lined with Teflon. The samples must be preserved with enough concentrated nitric acid (Baker Analyzed only) to bring the pH below 1, and enough saturated potassium dichromate solution to maintain a pale yellow colour.

Safety consideration.

Samples suspected to be high in halides should be acidified and digested in a fume hood for the protection of the analyst.

TOTAL MERCURY

Acid Digestion - Manual Flameless Atomic Absorption Method A

1. Introduction

A mixture of sulphuric and nitric acids is added to the water sample to break down organics. Potassium permanganate and potassium persulphate are then added to further oxidize the sample. The solutions, one containing stannous chloride, and the other hydroxylamine sulphate and sodium chloride are used to reduce the mercury to its atomic form.

After mercury reduction, a stream of air is bubbled through the solution using a vacuum system. The mercury vapor is carried into a flow-through absorption cell positioned between a source of 253.7 nm wavelength light and a detector which measures the degree of attenuation of the source light. This absorption is displayed on a recorder and the tracings are compared with those of known standards. The method described has application in the analysis of potable and surface waters, and industrial and sewage treatment plant effluents which contain extremely low levels of mercury.

2. Interferences and Shortcomings

Water vapor at a sufficiently high concentration in the system can condense on the windows of the absorption cell, causing diffraction of the radiation of the 253.7 nm resonance line of mercury. This problem is overcome by removing a sufficient amount of the water vapor so that condensation cannot occur. The insertion of a calcium sulphate trap reduces the vapor level.

Other interfering substances include hydrogen sulphide, aromatic hydrocarbons, carbon dioxide and other gases capable of absorbing light at 253.7 nm, but these are generally removed by an adequate sample preparation.

Samples suspected of containing high levels of chloride should be handled with extreme care, since toxic chlorine gas can evolve upon addition of sulphuric acid, nitric acid and potassium permanganate during the determination. The suspect sample should be placed in a fume hood and a portion of the sample neutralized to pH 7. This aliquot is then treated with starch and iodide test paper which will turn blue if chlorine is being evolved. Samples which react positively to the test should be analyzed by the sediment procedure.

3. Apparatus

- 3.1. Water bath; capable of maintaining a temperature of 85-90°C.
- 3.2. LDC Mercury Monitor; Model 1265, supplied by Pharmacia Ltd.
- 3.3. Air-Vacuum pump; Millipore, capable of delivering air flow of 3 l/min.
- 3.4. Recorder; Sargent-Welch, Model S.R.G.

- 3.5. Flowmeter; Gilmont (size No. 3).
- 3.6. Glass dispersion tube; with fritted glass cylinder, 250 mm length, stem diameter 8 mm.
- 3.7. Bottles; special bacteriological, calibrated at 100 ml. Bottles are cleaned by machine washing in distilled water.
- 3.8. Drying tube; containing calcium sulphate or magnesium perchlorate, change after every twenty samples.
- 3.9. Drechsel bottles.

4. Reagents

ALL REAGENTS SHOULD BE ANALYZED IN THE LABORATORY AND ONLY THOSE WITH A SUFFICIENTLY LOW MERCURY CONTENT SHOULD BE USED.

- 4.1. Sulphuric acid (H_2SO_4), concentrated, reagent grade. Must contain no measurable mercury content (Aristar recommended). Reagents other than those recommended in this section may possess the required degree of purity.
- 4.2. Nitric acid (HNO_3), concentrated reagent grade. Must contain no measurable mercury content (Baker Analyzed recommended).
- 4.3. Potassium permanganate (KMnO_4), reagent grade, Analar (BDH).
- 4.4. Potassium Persulphate ($\text{K}_2\text{S}_2\text{O}_8$), reagent grade, Analar (BDH).
- 4.5. Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), reagent grade crystals.
- 4.6. Sodium chloride (NaCl), reagent grade, Analar (BDH).
- 4.7. Stannous chloride (SnCl_2), reagent grade, Analar (BDH).
- 4.8. Hydroxylamine sulphate ($\text{NH}_2\text{OH} \cdot \text{SO}_4$) laboratory reagent (BDH).
- 4.9. Calcium sulphate (CaSO_4) laboratory reagent.
- 4.10. Mercuric chloride (HgCl_2), reagent grade, Mallinckrodt Chemicals.
- 4.11. Methylmercuric chloride (CH_3HgCl), reagent grade, Alfa Organics.
- 4.12. Methanol (CH_3OH), reagent grade, low mercury content.
- 4.13. Hydrochloric Acid (HCl) - 10%. Diluted 100 ml concentrated HCl to 1 liter with distilled, deionized water.
- 4.14. **Sodium Chloride-Hydroxylamine Sulphate Solution**
Dissolve 12 g sodium chloride and 12 g hydroxylamine sulphate in distilled water and make up to 100 ml.
- 4.15. **Stannous Chloride Solution (15% w/v)**
Dissolve 30 g stannous chloride in 10% hydrochloric acid dilute to a final volume of 200 ml with distilled, deionized water.

4.16. Potassium Permanganate Solution (saturated)

Dissolve 60 g of potassium permanganate in 1 liter distilled water. Allow solution to stand in sunlight for at least 48 hours before use in order to form small amounts of manganese dioxide, which slowly settle out. This step greatly reduces the risk of mercury contamination from the potassium permanganate.

4.17. Potassium Persulphate Solution (5% w/v)

Dissolve 5 g potassium persulphate in 100 ml distilled water.

4.18. Mercuric Chloride Stock Solution (1000 μ g/ml Hg)

Dissolve 1.358 g mercuric chloride in 1 liter of distilled water containing 10 ml concentrated nitric acid.

4.19. Methylmercuric Chloride Stock Solution (500 μ g/ml Hg)

Dissolve 0.6258 g methylmercuric chloride in 1000 ml methanol (reagent grade).

4.20. Mercury Standards

Make successive dilutions of the stock mercury solutions to obtain working standard solution containing 10 μ g/l mercury in the inorganic and organic forms.

4.21 Potassium Dichromate Solution (5% w/v)

Dissolve 5 g potassium dichromate in 100 ml distilled water.
This solution is used for sample preservation.

5. Procedure

- 5.1 Set up system as shown in Figure 1.
- 5.2. Transfer 1.0, 2.0 and 3.0 ml aliquots of the 10 μ g/l methylmercury working standard to the bacti bottles. Make up to 100 ml with distilled water and mix thoroughly. For samples, 100 ml aliquots are taken. If smaller aliquots are used, make to a final volume of 100 ml with distilled water.
- 5.3. Add 5.0 ml concentrated sulphuric acid and 2.5 ml concentrated nitric acid to each bottle. The addition of sulphuric acid generates heat, but with the stated amount of acid and a 100 ml aliquot the temperature rise is only about 13°C and no loss of mercury should occur.
- 5.4. Add 1 ml saturated potassium permanganate solution and 2 ml potassium persulphate solution to each bottle and mix well.
- 5.5. Heat samples (with caps loosened) for 2 hours at 85°C to 90°C in a water bath.
- 5.6. Remove samples from the water bath and cool to room temperature.
- 5.7. Add 1.5 ml sodium chloride-hydroxylamine sulphate solution to reduce the excess permanganate present in the samples.

- 5.8. Treating each bottle individually, transfer the sample to a drechsel bottle, add 5 ml stannous chloride solution and aerate the sample immediately by connecting to the vacuum FAAS system.
- 5.9. The atomic mercury (Hg^0) absorbs some of the light emitted by the UV lamp, and this absorption is displayed as a peak on a recorder. After the absorbance reaches a maximum and the recorder pen returns close to the original base line (zero absorbance), the drechsel bottle is removed and the system is allowed to come to equilibrium.
- 5.10. A standard curve is plotted from the absorption measurements of the standards that are run in parallel with the samples. The curve obeys Beer's Law up to 100 $\mu\text{g/l}$ and the majority of water samples are either in this range or can be diluted so as to fall within the workable portion of the curve.

6. Calculation and Reporting

The blank value is subtracted from the peak heights and a standard curve constructed using a least squares fit to the formula $y = mx$. The concentration of mercury in $\mu\text{g/l}$ in the sample is calculated from the sample peak heights and dilution factor:

$$\mu\text{g/l mercury} = \frac{y}{mx} \times \frac{100}{d}$$

Where:

d = aliquot taken

Reporting:

Range in $\mu\text{g/l}$	Reported in $\mu\text{g/l}$
0.05	0.05
0.05 to 0.09	1 significant figure
0.10 to 9.9	2 significant figures
10 to 100	2 significant figures

7. Precision and Accuracy

A series of samples received from EPA ranging from 0.2 to 10 $\mu\text{g/l}$ were analyzed by this method and the average recovery was found to be 99.3%. At the 0.4 $\mu\text{g/l}$ level the precision was 3.6%.

8. Bibliography

- 8.1. Bishop, J.N., Taylor, L.A. and Neary, B.P. (1973). The Determination of Mercury in Environmental Samples. Ontario Ministry of the Environment, Laboratory Services Branch, Toronto, Ontario.
- 8.2. Hatch, W.R. and Ott, W.L. (1968). Determination of submicrogram quantities of mercury by atomic absorption spectrophotometry. *Analytical Chemistry*, **40**:2085.
- 8.3. Uthe, J.F., Armstrong, F.A.J. and Stainton, M.P. (1970). Mercury determination in fish samples by wet digestion and flameless atomic absorption spectrophotometry. *Journal of the Fisheries Research Board of Canada* **27**: 805.

TOTAL MERCURY

Acid Digestion - Automated Flameless Atomic Absorption Method B

SUMMARY

Matrix.	This method is used for total mercury determinations on soil, sediment, sewage sludges, vegetation and biological material.
Substance determined.	Total mercury, Hg.
Interpretation of results.	Results are reported as $\mu\text{g/g}$ for sediments, soils, sewages, sludges, vegetation and biological material. Total mercury in fish is generally interpreted as being indicative of the methylmercury content, since methylmercury is usually 80 - 100% of the total mercury concentration.
Principle of method.	Samples are digested with acid while heating, cooled, brought to volume and transferred to an autoanalyzer where the mercury in an aliquot is reduced to elemental mercury by a reagent mixture of stannous chloride, hydroxylamine sulphate and sodium chloride in 10% hydrochloric acid. The elemental mercury is sparged into a cell where it is measured by flameless atomic absorption.
Time required for analysis.	For soils, vegetation and biological material, approximately 50 samples can be analyzed per day. A single sediment analysis requires approximately 1 day but samples can be done in batches of 20 samples in duplicate (plus blanks, standards and spikes).
Range of application.	0.01 - 2.5 $\mu\text{g/g}$ soils, vegetation and biological material. 0.01 - 1.6 $\mu\text{g/g}$ for undiluted sediment and sewage sludge samples.
Standard deviation.	For soils, vegetation and biological material the standard deviation of within-run duplicates is 3% (based on 148 samples), and 7% on 38 between-run duplicates. For sediments the standard deviation is 2% at 1 $\mu\text{g/g}$ level.
Accuracy.	For soils, vegetation and biological material spike recoveries of 97% on repeated determinations on 2.0 $\mu\text{g/g}$ Kodak Gelatin are found. For sediments using the aqua regia digestion, 96% recovery at the 0.8 $\mu\text{g/g}$ mercury level and 102% at the 0.4 $\mu\text{g/g}$ level are obtained.

Detection criteria.	For soils, vegetation and biological material, using a 0.3 g sample weight, the detection criteria is about 0.01 $\mu\text{g/g}$. Electronic scale expansion or larger sample lines can be used to increase sensitivity.
Interferences and shortcomings.	Selenium, tellurium, antimony, bismuth and arsenic are known interferents but are rarely present in concentrations great enough to cause problems. Organic solvents, NO_2 and Cl_2 will interfere by absorbing at 253.7 nm.
Minimum volume of sample.	5 grams dried soil, sediment, sewage sludge, vegetation or ground fish tissue.
Preservation and sample container.	Sediments are collected in glass jars with non-metallic lined caps. Soil samples are collected using a 2 cm (3/4") stainless steel tube and stored in plastic bags or glass jars with non-metallic lined caps. Vegetation samples are placed in perforated plastic bags. Fish samples are stored in small glass vials, capped, and frozen.
Safety considerations.	Extreme care must be taken when handling concentrated acids and the aqua regia mixture. Exothermic reactions during some stages of the method will occur.

TOTAL MERCURY

Acid Digestion - Automated Flameless Atomic Absorption Method B

1. Introduction

Soil, vegetation and biological material (usually fish) samples are digested in a 4:1 volumetric sulphuric:nitric acid mixture and heated in an aluminum block on a hot plate for a minimum of 6 hours.

Sediment samples are digested in aqua regia and potassium permanganate on a hot plate at approximately 100 °C. After cooling, hydroxylamine sulphate solution is added to reduce any excess permanganate.

All samples are placed in an autoanalyzer and reacted with stannous chloride, hydroxylamine sulphate and sodium chloride in 10% hydrochloric acid. This converts mercuric ions to elemental mercury which is then sparged by an air stream into an absorption cell where the mercury concentration is measured by atomic absorption of UV light at 253.7 nm.

2. Interferences and Shortcomings

There are a number of elements and their oxides which can interfere both chemically and/or spectrally with mercury when analyzed by FAAS. Selenium, tellurium, antimony, bismuth and arsenic are reported to interfere chemically by oxidizing the stannous chloride used as a reducing agent, but this effect has not been observed in this laboratory, perhaps because these elements occur in insufficient amounts to cause problems. Interfering substances causing positive spectral interference include NO_2 , Cl_2 , water vapor and any volatile organic solvents which absorb light at 253.7 nm. NO_2 , CO_2 and Cl_2 should not be present after the digestion step. Organic solvents (e.g. acetone and chloroform) should not be used for cleaning glassware. Water vapor is prevented from condensing in the system by preceding the cell with a heated trap and wrapping the optical cell with heating tape.

3. Apparatus

3.1 All Samples

- 3.1.1. Laboratory Data Control mercury monitor (see FAAS Apparatus - Figure 1).
- 3.1.2. Recorder (soils, vegetation and biological material: Brinkman; sediment: Sargent Welch (Model SRG)).
- 3.1.3. Analytical balance, Sartorius top loading, 4 place electronic, automatic tare.
- 3.1.4. Hot plate, Lindberg, capable of 300 °C.
- 3.1.5. Technicon autoanalyzer fittings and tubes (see Figure 2).

- 3.1.6. Gas separator and trap (transfer bulb) (see Figures Hg 3 and Hg 4).
- 3.1.7. Flow meter, 0 - 100 ml/min.
- 3.1.8. Gilson peristaltic pump and autosampler.
- 3.1.9. Heating tape.
- 3.1.10. Variac variable rheostats.
- 3.1.11. Gas bubble counter used as water droplet trap.

3.2. Soils, Vegetation and Biological Material

- 3.2.1. Culture tubes, Pyrex, 50 ml.
- 3.2.2. Aluminum hot blocks.

3.3. Sediments

- 3.3.1. Beakers, Phillips, conical, Pyrex with spout, 125 ml (Corning 1280).
- 3.3.2. Evaporating dish.
- 3.3.3. Filter paper, Whatman, No. 4 Qualitative (12.5cm).
- 3.3.4. Funnels, fluted, accurate 60°C, 65 mm I.D., 150 mm long stem, strong beaded edge.
- 3.3.5. Culture tubes, Pyrex, 100 ml.

3.4. Cleaning of Apparatus

- 3.4.1. The optical cell should be cleaned once a week and/or following contamination with excessively high level mercury samples. Rinse cell with methanol.
NOTE: Cell should then be blown dry with air.
- 3.4.2. Funnels and Phillips beakers are cleaned immediately after use with distilled water.
- 3.4.3. All glassware and apparatus must be thoroughly cleaned prior to use.
- 3.4.4. All polyethylene and glass tubing should either be replaced or rinsed with water followed by methanol and blown dry with a stream of air.

4. Reagents

ALL REAGENTS USED SHOULD BE ANALYZED IN THE LABORATORY AND ONLY THOSE WITH A SUFFICIENTLY LOW MERCURY CONTENT RETAINED.

4.1. All Samples

- 4.1.1. Nitric acid (HNO_3), concentrated, reagent grade. Of several brands investigated only Baker Analyzed reagent has been found to be satisfactory.
- 4.1.2. Hydrochloric acid (HCl), concentrated, reagent grade, Baker analyzed has been found to be satisfactory.
- 4.1.3. Hydroxylamine
- 4.1.4. Stannous chloride (SnCl_2), analytical reagent grade, BDH Analar reagent is acceptable.
- 4.1.5. Mercuric chloride (HgCl_2), reagent grade as supplied by Mallinckrodt Chemicals.
- 4.1.6. Methylmercuric chloride, (CH_3HgCl), reagent grade, Alpha Organics is acceptable.
- 4.1.7. Methanol (CH_3OH), reagent grade, low mercury content.
- 4.1.8. **Reductant Solution**

Combine the following reagents in the order given in a 1 liter Erlenmyer flask:

200 ml distilled water
100 ml hydrochloric acid conc.
40 g stannous chloride
20 g hydroxylamine sulphate
10 g sodium chloride

Mix well, bring to 1 liter with distilled water and mix again. There is usually some undissolved material in the flask, but it does not affect the system. Filtration is therefore unnecessary.

4.1.9. **Mercuric Chloride Stock Solution (1000 $\mu\text{g}/\text{ml}$)**

Dissolve 1.358 g mercuric chloride in 1 liter distilled water containing 10 ml concentrated nitric acid.

4.1.10. **Methylmercuric Chloride Stock Solution (500 $\mu\text{g}/\text{ml}$)**

Dissolve 0.6258 g methylmercuric chloride in 1000 ml methanol (reagent grade).

4.1.11. Mercury Standards (1 µg/ml Hg)

Make successive dilutions from the stock solution in distilled water. New working solutions should be made up weekly and the concentration of mercury must be within 1% of the old.

4.1.12 Sulphuric Acid Wash Solution (15%)

Dilute 150 ml concentrated sulphuric acid to 1000 ml with distilled water.

4.2. Sediments Only

4.2.1. Aqua Regia

Prepare a volumetric ratio of 4 parts distilled water, 3 parts concentrated hydrochloric acid and 1 part concentrated nitric acid. Caution should be exercised during preparation, as this mixture has an exothermic reaction upon mixing.

4.2.2. Potassium Permanganate Solution (saturated)

Dissolve 60 g potassium permanganate in 1 liter distilled water at room temperature. The solution should be allowed to stand for at least 48 hours in sunlight before use, in order to form a small amount of manganese dioxide which slowly settles out. This step greatly reduces the risk of mercury contamination from the potassium permanganate.

4.2.3. Hydroxylamine Sulphate Solution (20% w/v)

Dissolve 200 g reagent grade hydroxylamine sulphate in 1 liter distilled water.

NOTE: THIS SUBSTANCE IS CARCINOGENIC, AVOID CONTACT WITH THE SKIN.

4.2.4. Stannous Chloride Solution (20% w/v)

Dissolve 400 g reagent grade stannous chloride in 2 liters concentrated hydrochloric acid.

4.3. Soils, Vegetation and Biological Material Only

4.3.1 Sulphuric-Nitric Acid (4:1 v/v)

Combine 800 ml concentrated sulphuric acid and 200 ml concentrated nitric acid.

5. Procedure

5.1. Digestion - Soils, Vegetation and Biological Material

5.1.1. Soil samples should have previously been air dried, ground sieved and stored in glass bottles.

Vegetation samples should have been washed, dried at 80 °C, sieved through 40 mesh screen and stored in glass bottles.

Thaw biomaterials and place 5 g into a clean stainless steel cup. Grind tissue for 2 minutes with mechanical homogenizer and transfer 2 - 5 g to a small glass vial, using a stainless steel spatula. Freeze samples until analyzed.

- 5.1.2. Weigh a 0.25 g sample into a tared 50 ml Pyrex tube and record weight to the nearest 0.001 g.

NOTE: Thaw biomaterials prior to weighing and homogenize again with spatula, ensuring that the liquid-flesh partition, which occurs during freezing, does not cause imprecise or inaccurate results.

- 5.1.3. In addition to samples, prepare:

5.1.3.1. an undigested control (UND).

5.1.3.2. 2 reagent blanks (BL).

5.1.3.3. 0.1, 0.2, 0.3, 0.4, 0.5 µg mercury standards prepared from a 1 µg/l mercury solution as methylmercuric chloride.

5.1.3.4. a duplicate control sample (CONT) composed of a large, composite dried, homogenized sample which has been analyzed at least 20X prior to use as a control.

5.1.3.5. every 20th sample in triplicate and spike 1 of these replicates with 0.2 µg methylmercury (DUP + SP).

- 5.1.4. Add 5 ml of 4:1 v/v sulphuric-nitric acid mixture to each tube.

- 5.1.5. Place tubes in an aluminum block which is heated to 230°C for at least 6 hours.

- 5.1.6. Cool the clear, colorless or pale yellow solutions and bring close to the mark with distilled water.

NOTE: YOU ARE ADDING WATER TO CONCENTRATED ACID. THE REACTION IS VERY EXOTHERMIC AND MAY CAUSE SPATTERING.

- 5.1.7. Bring to 25 ml and mix samples well on a Vortex mixer.

- 5.1.8. Pour samples into labelled tubes compatible with the autosampler. Analyze according to 5.3.

5.2. Digestion - Sediments and Sewage Sludge

- 5.2.1. Sediments are not dried prior to analysis. Decant excess liquid from the sample bottle and homogenize the sample with a glass rod. Manually remove portions of the homogenate with a scoopula, taking care to exclude sticks, large stones (exceeding 5 mm in diameter), leaves and other extraneous material.

Sewage sludge samples are stirred with a wide mouth sludge pipette and a 10 ml aliquot taken. Digest as described in 5.2.4. to 5.2.10.

- 5.2.2. Weigh duplicate portions of 2 to 4 grams of the selected homogenate into a tared 125 ml Phillips beaker using a single pan analytical balance, accurate to 4 significant figures. Record the weight of the sample.

- 5.2.3. At the same time weigh approximately 5 g of the homogenate into a tared evaporating dish. Record the weight of sample and dish and dry the sample at 110°C for a minimum of 3 hours. After drying to constant weight, reweigh the sample and dish and derive a factor that indicates the relationship between the weight of the dried sample and

wet sample. This factor is eventually applied to the weight of the sample taken for analysis to obtain the mercury concentration on a dry weight basis (Section 6.2).

5.2.4. In addition to the samples, the run consists of:

5.2.4.1. 2 reagent blanks (BL)

5.2.4.2. 0.3, 0.5, 1.0 and 2.0 μg organic mercury standards (ST)

5.2.4.3. duplicate aliquots of a control sediment (CONT)

5.2.4.4. at least 2 samples analyzed in triplicate with 1 spiked aliquot (DUP + SP).

The standards are analyzed before and after the samples in order to monitor sensitivity changes during the run. All samples with apparent or suspected inhomogeneity are analyzed in duplicate.

5.2.5. To each sample add a few antibumping granules and 10 ml aqua regia digestion acid.

5.2.6. Place the digestate on a hot plate at medium heat, swirl the samples to prevent bumping and foaming, and heat for five minutes.

NOTE: DO NOT ALLOW SAMPLES TO BOIL.

5.2.7. Remove samples from hot plate and allow to cool.

5.2.8. Wash down beaker sides with 35 ml distilled water. Add 15 ml saturated potassium permanganate solution. Reheat samples to incipient boiling and digest for 45 minutes. Do not allow bumping of samples to occur. Remove samples from hotplate and cool.

5.2.9. Add approximately 3 ml hydroxylamine sulphate (20% w/v) to reduce excess permanganate and manganese dioxide.

NOTE: This step should not be completed unless samples can be analyzed within the next hour.

5.2.10. Filter samples through Whatman No. 4, qualitative (12.5 cm) filter paper into 100 ml bottles and make to volume with distilled water. Analyze according to 5.3.

5.3. Automated Flameless Atomic Absorption

REFER TO MANUFACTURER'S INSTRUCTION MANUAL FOR SET-UP, OPERATING, CHECKING AND CLEANING PROCEDURES FOR AUTOANALYZER AND LDC MERCURY MONITOR.

5.3.1. Set up system as in Figure Hg 2. The cell in the mercury monitor is wrapped with heating tape connected to a Variac and is maintained at approximately 80 °C to prevent the condensation of water vapor.

5.3.2. The loading sequence for the autoanalyzer is UND, BL, STN, CONT., DUP + SP, 20 SAMPLES, DUP + SP, UND, 20 SAMPLES, DUP + SP, UND ST, UND.

5.3.3. Start run. Atomic absorption of UV light is measured at 253.7 nm. Sensitivity changes of more than 10% during a run are not tolerated. If this occurs, locate problem and re-run.

- 5.3.4. Dilute offscale samples with 15% sulphuric acid and re-run. Dilutions may also be accomplished by altering the manifold (Figure Hg 2).
- 5.3.5. Subtract blank values from all peak heights and construct a calibration curve using a least squares program employing the peak heights of the digested standards.

6. Calculation and Reporting

Samples concentrations are calculated from the calibration curve (5.3.4.).

- 6.1. For soil, vegetation and biological materials $\mu\text{g/g}$ mercury in the sample is determined as follows:

$$\mu\text{g/g mercury} = \frac{a}{w} \times d$$

Where:

a = mg/l mercury in sample solution
w = sample weight
d = dilution factor

For results below 0.1 mg/g report to 1 significant figure.
For results above 0.1 $\mu\text{g/g}$ report to 2 significant figures.

- 6.2. For sediment samples $\mu\text{g/g}$ mercury in the sample is determined as follows:

$$\mu\text{g/g mercury (dry weight)} = \frac{d}{w} \times a$$

Where:

a = mg/l mercury in sample solution
w = weight of wet sample
d = drying factor = $\frac{\text{weight wet sample}}{\text{weight dry sample}}$

Reporting

Range in $\mu\text{g/g}$	Reported in $\mu\text{g/g}$
<0.01	<0.01
0.01 to 0.09	1 significant figure
0.10 to 0.99	2 significant figures
1.0 to 99	2 significant figures

7. Precision and Accuracy

7.1. Soil, Vegetation and Biological Material

The relative standard deviations for within-run and between run duplicate analyses are 3% and 7% respectively. Recoveries of 97-98% are obtained on spiked samples and kodak gelatin (2.0 $\mu\text{g/g}$) samples.

7.2. Sediment Samples

At the 1 $\mu\text{g/g}$ mercury level the standard deviation is 2%. Mercury recovery from spiked sediments digested in aqua regia is 96% at 0.8 $\mu\text{g/g}$ and 102% at the 0.4 $\mu\text{g/g}$ level.

8. Bibliography

- 8.1. Bishop, J.N., Taylor, L.A. and Neary, B.P. (1973). The Determination of Mercury in Environmental Samples. Ontario Ministry of the Environment, Laboratory Services Branch, Toronto, Ontario.
- 8.2. Hatch, W.R. and Ott, W.L. (1968). Determination of submicrogram quantities of mercury by atomic absorption spectrophotometry. *Analytical Chemistry*, **40**:2085.
- 8.3. Uthe, J.F., Armstrong, F.A.J. and Stainton, M.P. (1970). Mercury determination in fish samples by wet digestion and flameless atomic absorption spectrophotometry. *Journal of the Fisheries Research Board of Canada*, **27**: 805.

METHYLMERCURY

Gas Chromatography Method C

SUMMARY

Matrix.	This method is used on water and biological samples (mostly fish muscle).
Substance determined.	Methylmercuric chloride, CH_3HgCl for water samples. Methylmercuric bromide, CH_3HgBr for biological samples.
Interpretation of results.	For water samples, results are reported as ng/l mercury as methylmercuric chloride. For biological samples results are reported as $\mu\text{g/g}$ mercury as methylmercuric bromide. Methylmercury results are calculated without a correction factor, however, studies using spiked samples have shown that $90 \pm 10\%$ methylmercury is recovered from water samples and $89 \pm 5\%$ is recovered from fish tissue.
Principle of method.	<p>For water samples, methylmercury is released from fine particulate or water soluble complexes by the addition of sulphuric acid. The addition of sodium chloride produces benzene soluble methylmercuric chloride. A benzene extraction is performed, the methylmercuric chloride is concentrated and back extracted into aqueous L-cysteine hydrochloric acid solutions. The methylmercuric chloride is extracted into benzene and measured by gas chromatography.</p> <p>For fish samples, methylmercury is released from the muscle protein complex by the addition of copper sulphate and sodium bromide in acid, and is extracted as the bromide in toluene. Cleanup is achieved by methylmercury extraction as the thiosulphate complex into aqueous sodium thiosulphate solution. The complex is broken by adding cupric bromide and the methylmercuric bromide formed is extracted into benzene and analyzed by gas chromatography.</p>
Time required for analysis.	For water samples a single analysis requires 2 days, however, 4 samples can be analyzed simultaneously. For fish samples 1-½ days are required for a single analysis, however, 20 or 30 samples can be run simultaneously.
Range of application.	1 ng/l as methylmercuric chloride is detectable in 4 liters of water. Higher concentrations may be analyzed by dilution. For fish samples 0.05 $\mu\text{g/g}$ Hg as methylmercuric bromide is detectable on 1 g fish muscle. Higher concentrations may be analyzed after dilution.

Standard deviation.	15% relative standard deviation on concentrations over 3 ng/l for water samples. For fish samples relative standard deviations of 10% are found on concentrations over 0.1 µg/g.
Accuracy.	90% ± 10% recovery of methylmercury on spiked water samples; 89% ± 5% methylmercury recovery on spiked fish tissue.
Detection criteria.	For 4 liter water samples, detection criteria = 1 ng/l. For 1 g fish samples, detection criteria = 0.05 g, this can be lowered by using a larger sample.
Interferences and shortcomings.	There are no known interferences with this method. Prolonged exposure to light during sample extraction can cause low, variable results due to photochemical breakdown of methylmercury by light. In aqueous samples results may also reflect methylmercury on fine suspended material rather than only the fraction in solution.
Minimum volume of sample.	4 liters of water is required to detect concentrations as low as 1 ng/l. In fish tissue 1 g is required to detect concentrations as low as 0.05 µg/g.
Preservation and sample container.	Water samples should be preserved with 2 ml concentrated nitric acid per liter of water. Glass bottles with plastic caps should be used for sample storage. Analyze samples within 10 days. Fish muscle should be frozen and protected from light during storage.
Safety considerations.	Normal laboratory safety precautions.

METHYLMERCURY

Gas Chromatography Method C

1. Introduction

In water samples, the methylmercuric ion is liberated from water soluble complexes or suspended particulate by the addition of sulphuric acid. Sodium chloride is added producing benzene soluble mercuric chloride which is extracted into benzene. Back extraction into aqueous L-cysteine removes interferences and results in a methylmercury-cysteine complex. Methylmercuric chloride is once more formed by the addition of hydrochloric acid and extraction into benzene. Back extraction is repeated and the reduced final benzene layer is analyzed by gas chromatography using an electron capture detector.

Methylmercury is released from the protein complex in fish muscle by adding cupric sulphate and an acidic solution of sodium bromide. Interferences are removed by the extraction of methylmercury as its thiosulphate complex into aqueous sodium thiosulphate solution. The addition of cupric bromide breaks down this complex and methylmercuric bromide is extracted into benzene and measured by gas chromatography using an electron capture detector.

2. Interferences and Shortcomings

There are no known interferences with this method. For water samples, however, the results might not represent the methylmercury concentration in solution. Sample filtration is not employed since methylmercury is adsorbed on the filter, and therefore results may reflect methylmercury on suspended particulate matter.

3. Apparatus

3.1. All Samples

- 3.1.1. Culture tubes, glass, 10 ml, with Teflon lined screw caps (30).
- 3.1.2. Analytical balance, capable of weighing to 0.1 mg.
- 3.1.3. Assorted pipettes.
- 3.1.4. Recorder with 1 mV full scale deflection.
- 3.1.5. Gas chromatograph with a supply of ultra-pure nitrogen and a tritium electron capture detector.
- 3.1.6. Chromatographic column, 6' x 1/8" I.D., Pyrex glass column packed with 5% carbowax 20 m on 5% sodium bromide impregnated Varaport 30 (100 - 200 mesh).

3.2. Water Samples

- 3.2.1. Continuous liquid-liquid extractors capable of holding 1 liter of water (see Figures Hg 5 and 6).
- 3.2.2. Heating mantles for 500 ml flasks and variable rheostats.
- 3.2.3. Assorted graduated cylinders.
- 3.2.4. Teflon coated stirring bars and magnetic stirrers.
- 3.2.5. Separatory funnels with Teflon stopcocks 500 ml, 2 x 50 ml, 20 ml.

3.3. Fish Samples

- 3.3.1. Culture tubes, glass, 25 ml, with Teflon lined screw caps (30).
- 3.3.2. Centrifuge tubes, glass, 10 ml, with Teflon lined screw caps (30).
- 3.3.3. Centrifuge capable of accepting 50 ml culture tubes at 2,000 rpm.
- 3.3.4. Clinical centrifuge for 10 ml centrifuge tubes.
- 3.3.5. Pasteur pipettes, disposable.
- 3.3.6. Polytron Homogenizer.

3.4. Cleaning

All glassware should be cleaned in the following way:

- 3.4.1. Wash glassware thoroughly with alcoholic potassium hydroxide (Reagent 4.1.7.)
- 3.4.2. Rinse well with tap water.
- 3.4.3. Rinse twice with an aqueous solution of 0.1 M sodium thiosulphate (Reagent 4.3.11.)
- 3.4.4. Rinse twice with distilled water.
- 3.4.5. Rinse with acetone and air dry.

4. Reagents

ALL REAGENTS SHOULD HAVE A SUFFICIENTLY LOW MERCURY CONTENT.

4.1. All Samples

- 4.1.1. Ethanol (C_2H_5OH), reagent grade.
- 4.1.2. Sulphuric acid (H_2SO_4), concentrated reagent grade.
- 4.1.3. Benzene, distilled in glass.
- 4.1.4. Potassium hydroxide (KOH), reagent grade pellets.

- 4.1.5. **Water.**
Each liter distilled water is extracted twice with 100 ml benzene.
- 4.1.6. **Ethanol (20% v/v)**
Dilute 200 ml ethanol to 1 liter with distilled water.
- 4.1.7. **Potassium Hydroxide Solution**
Dissolve 100 g potassium hydroxide in 1 liter 20% ethanol solution.
(Use this solution for cleaning glassware.)

4.2. Water Samples

- 4.2.1. Hydrochloric acid (HCl), concentrated, reagent grade, Baker Analyzed recommended.
- 4.2.2. Sodium chloride (NaCl), reagent grade, Analar (BDH).
- 4.2.3. Sodium acetate (CH_3COONa), anhydrous, reagent grade powder, ANALAR (BDH).
- 4.2.4. Sodium sulphate (NaSO_4), anhydrous, reagent grade powder, Analar (BDH).
- 4.2.5. Methylmercuric chloride (CH_3HgCl), reagent grade.
- 4.2.6. L-cysteine hydrochloride ($(\text{CH}_2\text{SH})\cdot\text{CH}(\text{COOH})\cdot\text{NH}_2\text{HCl}$), reagent grade.
- 4.2.7. **L-Cysteine Solution**
Dissolve 1 g L-cysteine hydrochloride, 0.98 g anhydrous sodium acetate and 12.5 g anhydrous sodium sulphate in 100 ml benzene washed distilled water.
- 4.2.8. **Methylmercuric Chloride Stock Solution (1000 mg/l Hg)**
Dissolve 0.6258 g methylmercuric chloride in 500 ml benzene. Dilute to 1 ng/l for gas chromatography calibration.

4.3. Fish Samples

- 4.3.1. Cupric bromide (CuBr_2), reagent grade powder.
- 4.3.2. Cupric sulphate ($\text{CuSO}_4\cdot 5\text{H}_2\text{O}$), reagent grade powder, Analar.
- 4.3.3. Sodium bromide (NaBr), reagent grade, Suprapur.
- 4.3.4. Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$), anhydrous, reagent grade powder.
- 4.3.5. Toluene ($\text{C}_6\text{H}_5\text{CH}_3$), reagent grade.
- 4.3.6. Sodium carbonate (NaCO_3), anhydrous reagent grade powder.
- 4.3.7. Methylmercuric bromide (CH_3HgBr), reagent grade, powder.
- 4.3.8. **Cupric Bromide Solution**
Dissolve 22.34 g cupric bromide in 100 ml distilled water and extract twice with 150 ml benzene. Prepare fresh solution daily.
- 4.3.9. **Cupric Sulphate Solution**
Dissolve 250 g cupric sulphate in 1 liter distilled water. Prepare fresh solution weekly and store in the dark.

4.3.10. Sodium Bromide Solution

Slowly add 110 ml concentrated sulphuric acid to 110 ml distilled water. Dissolve 310 g sodium bromide in 700 ml distilled water. Combine solutions and make up to 1 liter with distilled water. Prepare fresh solution weekly.

4.3.11. Sodium Thiosulphate Solution

Dissolve 12.4 g sodium thiosulphate in 1 liter distilled water. Add 100 mg anhydrous sodium carbonate. Dilute to 1:10 with distilled water. Prepare fresh solution bi-weekly.

4.3.12. Methylmercuric Bromide Stock Solution (1000 mg/l Hg)

Dissolve 1.4731 g methylmercuric bromide in 1:1 benzene. Dilute with benzene to 10 ng/l for gas chromatography calibration.

5. Procedure

5.1. Extraction - Water Samples

5.1.1. Pour sample into graduated cylinder, note volume and pour into continuous extractor.

5.1.2. Place stirring bar in sample and place extractor on magnetic stirrer.

5.1.3. Slowly add 35 ml concentrated sulphuric acid.

NOTE: DUE TO THE EXOTHERMIC REACTION RESULTING FROM THE ADDITION OF SULPHURIC ACID, THIS REAGENT SHOULD BE ADDED VERY SLOWLY AND ALLOWED TO MIX THOROUGHLY.

5.1.4. Add approximately 2 g sodium chloride.

5.1.5. Attach side arm and 500 ml round-bottom flask to extractor and insert second stage of extractor.

5.1.6. Pour 300 ml benzene into extractor through fritted glass tube as tube is lowered into the extractor.

5.1.7. Add distilled water until half of the benzene is in the boiling flask and half remains in the extractor and bubbling tube.

5.1.8. Fit condenser into top of extractor and turn on cold water.

5.1.9. Turn up variable rheostat so that benzene in flask begins to boil.

5.1.10. Allow extraction apparatus to run for 6 hours.

5.1.11. Recover 250 ml benzene from extractor and place in a 500 ml separatory funnel with a Teflon stopcock. Extract benzene 4 times with 5 ml portions of 1-cysteine solution.

5.1.12. Combine 1-cysteine layers and transfer to a 50 ml separatory funnel with Teflon stopcock.

5.1.13. Add 5 ml hydrochloric acid and extract cysteine solution 4 times for 2 minutes with 5 ml benzene.

- 5.1.14. Combine benzene layers and transfer to a 50 ml separatory funnel with Teflon stopcock.
- 5.1.15. Extract benzene twice with 2 ml aliquots of l-cysteine solution.
- 5.1.16. Combine l-cysteine solutions and place in a 20 ml separatory funnel with a Teflon stopcock.
- 5.1.17. Add 2 ml hydrochloric acid and extract cysteine layer twice for 2 minutes with 1 ml benzene.
- 5.1.18. Transfer benzene to 10 ml screw cap glass culture tube. Cap tightly and refrigerate until analyzed by gas chromatography (Step 5.3).
NOTE: Analyze extracts as quickly as possible. Extraction should be repeated if extracts have been standing more than 3 days.

5.2. Extraction - Fish

- 5.2.1. Thaw fish and place 5 g tissue in a stainless steel cup and grind for 2 minutes using a mechanical homogenizer. Freeze until ready to analyze.
- 5.2.2. Weigh approximately 1 g homogenized fish muscle into a numbered 25 ml screw-capped culture tube and add 5 ml extracted distilled water.
- 5.2.3. Homogenize fish samples in water for 1 minute on Polytron homogenizer. Wash homogenizer probe into tube with 5 ml distilled water. Clean probe between samples with distilled water.
- 5.2.4. Add 1 ml cupric sulphate solution and 5 ml sodium bromide solution to each tube. Cap tubes and allow to stand overnight in the dark.
- 5.2.5. Add 10 ml toluene to each tube, cap tubes and agitate manually for 2 minutes.
- 5.2.6. Balance tubes and centrifuge for 5 minutes at 2,000 rpm.
- 5.2.7. Using a Pasteur pipette and a syringe, transfer 5 ml of the toluene layer into a 10 ml graduated centrifuge tube. Discard pipette after each sample.
- 5.2.8. Add 2 ml sodium thiosulphate to the toluene extract and cap tubes. Shake manually for 2 minutes and centrifuge.
- 5.2.9. Using a Pasteur pipette and syringe, quantitatively transfer the thiosulphate layer to a 10 ml screw-cap culture tube. Discard pipette after each sample.
- 5.2.10. Add 2 ml cupric bromide solution and 1 ml benzene to the thiosulphate layer. Cap tubes and agitate for one minute.
- 5.2.11. Using a Pasteur pipette and syringe, transfer benzene layer to a 10 ml screw-cap tube. Cap tightly and store in a dark refrigerator until ready to analyze by gas chromatography (Step 5.3).

NOTE: The final benzene extracts should be analyzed as quickly as possible. If extracts have been left for more than 3 days the extraction should be repeated.

5.3. Gas Chromatographic Analysis

5.3.1. Operating Conditions (water and fish samples).

5.3.1.1. Gas flow: nitrogen (ultra-pure): 45 ml/min.

5.3.1.2. Injector temperature: 175°C.

5.3.1.3. Oven Temperature: 140°C.

5.3.1.4. Detector: e.c. (250 mC: ³H); 180°C.

5.3.2. Calibration of machine for water samples is done by injecting varying amounts of a 1 ng/l methylmercuric chloride solution. Calibration for fish samples is done by injecting 10 ng/l methylmercuric bromide into the machine.

5.3.3. A calibration curve is plotted and the samples are compared to the peak heights.

6. Calculation and Reporting

6.1. Mercury concentrations of water samples are reported as ng/l according to the following equation:

$$\text{ng/l Hg as methylmercuric chloride} = \frac{a \times 1.2 \times 2}{b \times c}$$

Where:

a = number of nanograms corresponding to peak height on the chromatogram

b = number of microliters of benzene layer injected into gas chromatograph

c = volume of sample taken

2 = final volume of benzene (ml)

1.2 arises because only 250 of the 300 ml benzene added to extractor are actually processed.

6.2. Mercury concentrations of fish samples are reported as µg/g according to the following equation:

$$\mu\text{g/g Hg as methylmercuric bromide} = \frac{a \times b \times 2}{c \times d \times 10^3}$$

Where:

a = number of nanograms corresponding to peak height on the chromatogram.

b = dilution factor

c = number of microliters of benzene injected into the gas chromatograph

d = weight of fish taken (g)

2 = amount of toluene layer taken

10³ converts the result in ng/g to µg/g

All results are reported to 2 significant figures.

7. Precision and Accuracy

For water samples, relative standard deviations of 15% are found on concentrations of greater than 3 ng/l methylmercuric chloride. For fish samples relative standard deviations of 10% are found on concentrations of over 0.1 µg/g.

Recoveries of 89% methylmercury are found on spiked fish muscle samples or spiked aqueous samples. This recovery factor agrees well with the theoretical maximum of 90% and with experimental values found by others. For water samples approximately 90% of the methylmercury is recovered.

8. Bibliography

- 8.1. Bishop, J.N., Taylor, L.A. and Neary, B.P. (1973). The Determination of Mercury in Environmental Samples. Ontario Ministry of the Environment, Laboratory Services Branch, Toronto, Ontario.
- 8.2. Westoo, G. (1967). The determination of methylmercury compounds in foodstuffs (1). *Acta Chemica Scandania* **20**: 2131.
- 8.3. Westoo, G. (1966). Determination of methylmercury compounds in foodstuffs. II Determination of methylmercury in fish, egg, meat and liver. *Acta Chemica Scandania* **21**: 1790.

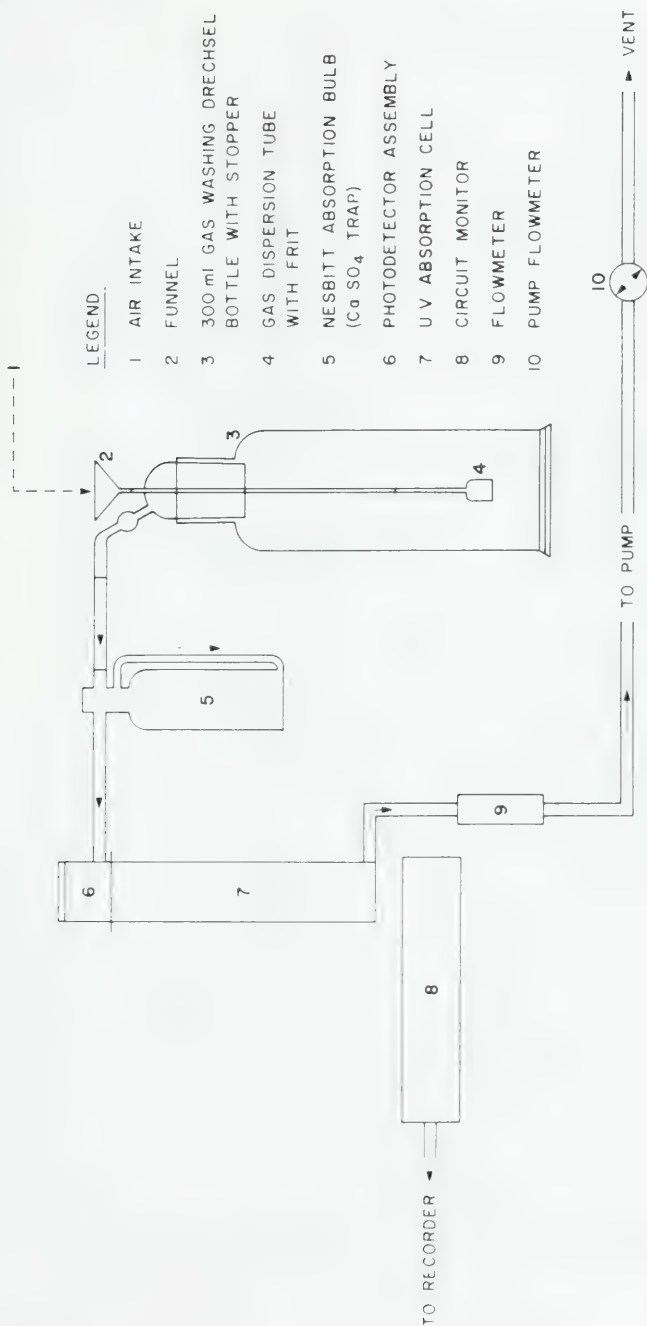


FIGURE 1 — APPARATUS FOR MERCURY DETERMINATION METHOD A

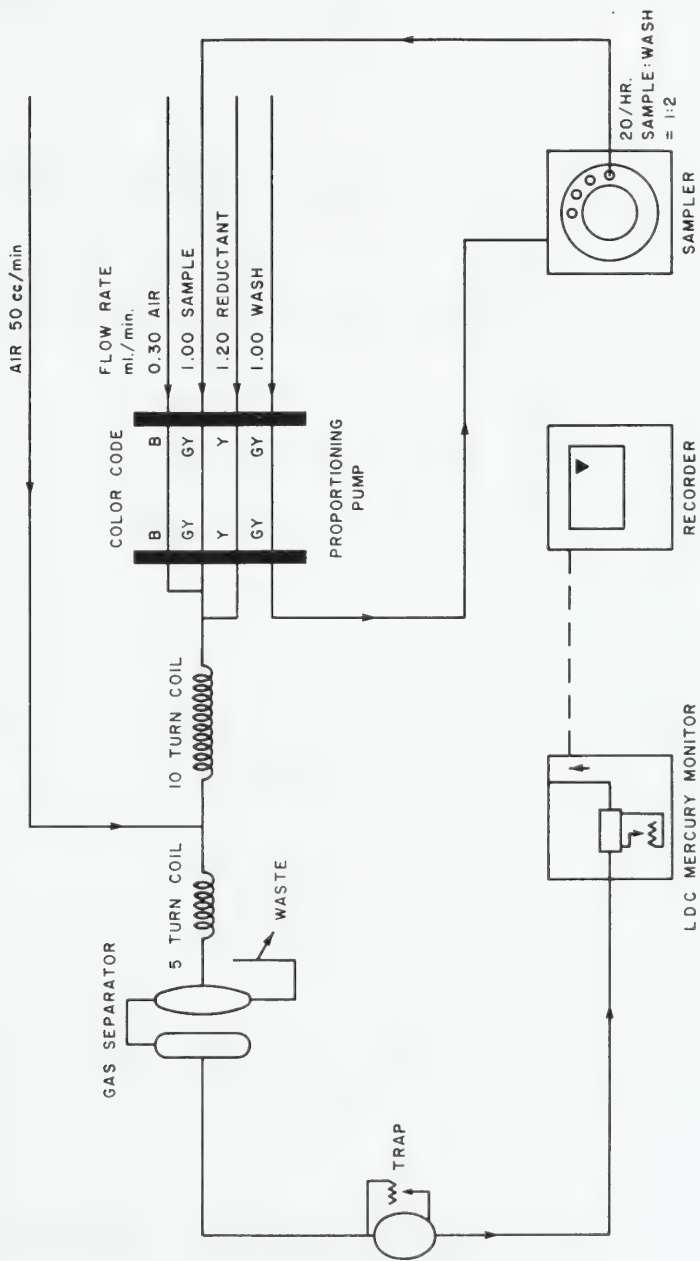


FIGURE 2 — AUTOMATED COLD VAPOR FLAMELESS ATOMIC ABSORPTION SYSTEM FOR MERCURY DETERMINATION

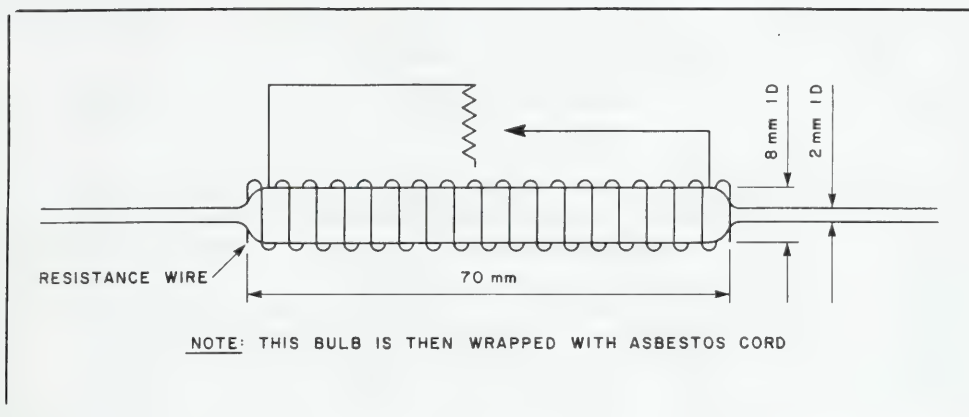


FIGURE 3 — HEATED TRANSFER BULB FOR AUTOMATED MERCURY DETERMINATION

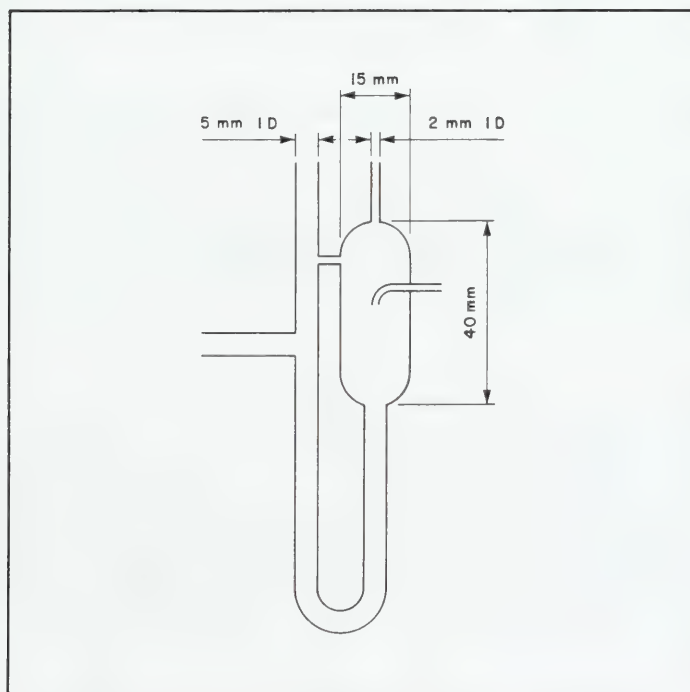
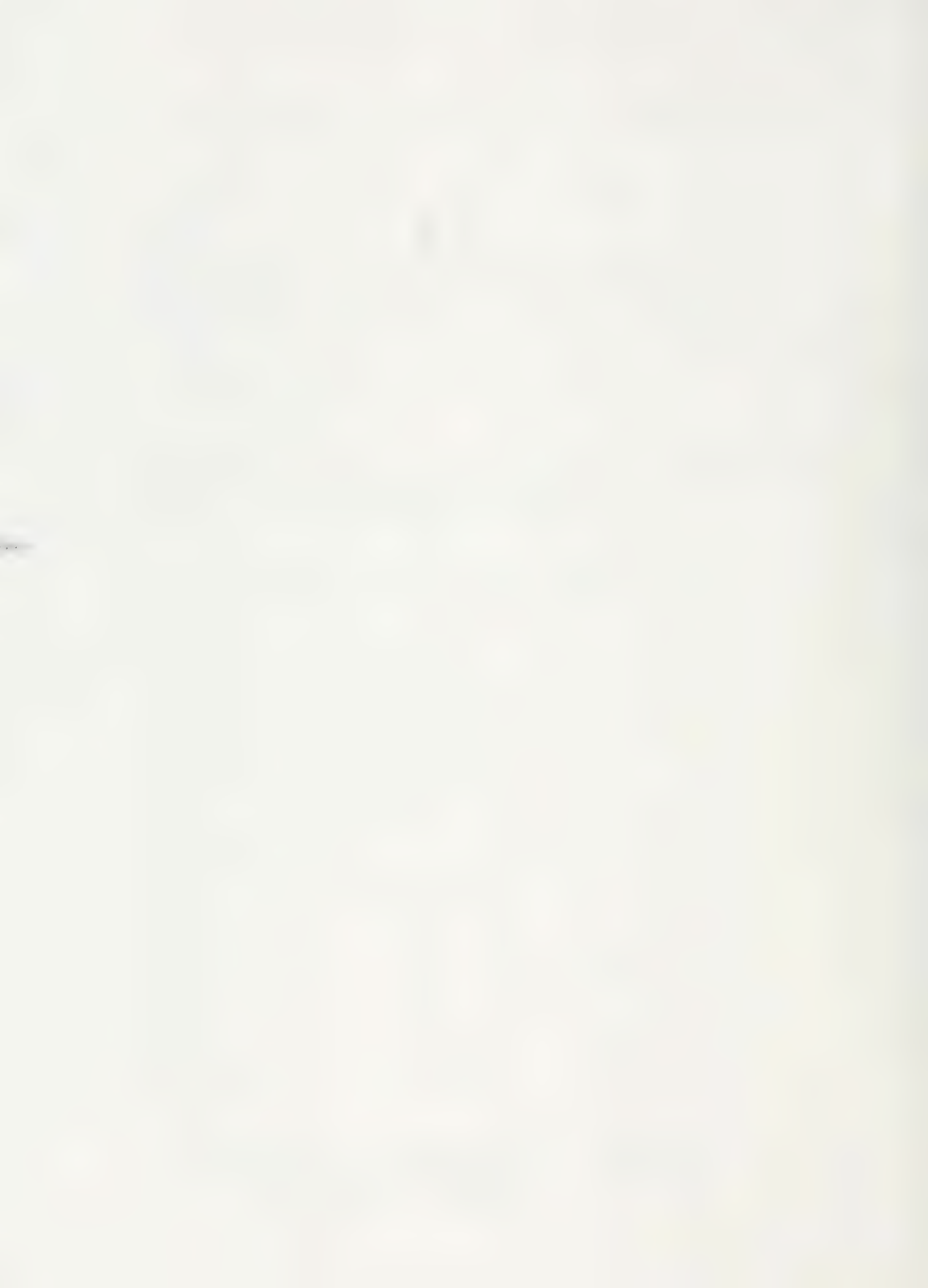


FIGURE 4 — GAS SEPARATOR FOR AUTOMATED MERCURY DETERMINATION



THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

There are approximately 60 elements which fall under the generic heading of "trace metals." In fact some, such as boron, are not metals at all, and in certain samples others, such as calcium and aluminum, can hardly be considered trace.

For the purposes of this procedure, trace metals will be considered to be those elements which are determinable at environmental levels by atomic absorption or atomic emission spectroscopy. Other analytical procedures employed for trace metal analysis will be discussed more fully under Selection of Method. Many other elements can be determined with adequate sensitivity by atomic spectroscopy but there is little current environmental interest in these elements at this time.

The elements determined by spectroscopic procedures in this laboratory are:

Routine

aluminum, calcium, cadmium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, potassium, sodium, vanadium, zinc (boron, arsenic, selenium).

Nonroutine

barium, beryllium, lithium, silver, strontium, thallium, titanium.

Infrequent

bismuth, gallium, germanium, gold, indium, zirconium.

The elements in brackets can be determined with adequate sensitivity for most matrices by atomic emission but are routinely analyzed by other procedures at this time.

ALUMINUM

Aluminum is one of the most abundant elements in nature and is widely distributed in both soluble and insoluble forms. The native metal and its oxides are insoluble, but many salts are highly soluble. Its most important sources include bauxite, cryolite, alum, corundum and kaolin minerals. Aluminosilicate minerals are abundant in igneous and sedimentary rocks and soils. As the minerals weather, aluminum may be left as an insoluble residue such as bauxite. Aluminum compounds occur naturally in water, but may also be derived from industrial wastes or effluents from water treatment plants where alum is commonly used as a precipitating or flocculating agent. Aluminum concentrations are generally less than 1.0 mg/l; however, acid mine drainage may lead to increased levels in certain areas. While aluminum is amphoteric, it is seldom found in the anionic form.

Aluminum is used extensively in domestic utensils, wire, exterior building finishes and in any industrial applications where a strong, light, easily constructed material is needed. Aluminum is also used in dye and paper manufacturing and in the tanning industry. The presence of small amounts of aluminum in water may interfere with certain industrial processes.

Oral toxicity of aluminum and aluminum salts seems to be negligible although ingestion of large amounts seems to alter the body's method of inorganic phosphate elimination. Inhalation of the element or its salts may cause lung problems. Toxic effects on plants have been reported for extreme conditions at both ends of the pH scale, but at a neutral pH, toxicity effects are negligible. Since aluminum has not been shown to be harmful to human health, no drinking water guidelines have been proposed. A limit of 0.100 mg/l has been proposed to protect aquatic biota. The Water Quality Criteria for livestock watering is 5.0 mg/l.

BARIUM

Barium is commonly found in nature as the minerals barite (barium sulphate) and witherite (barium carbonate). It is also found in igneous rock, primarily feldspar, where it is known to replace potassium. Barium ions in solution are rapidly precipitated by sulphate and carbonate. Most other barium salts are soluble. As a result, only trace levels are normally found. Weathering and industrial discharges are the main sources of barium in natural waters. Industrial application of barium includes the manufacture of metal alloys, paints, pigments, paper, soap, rubber, linoleum, flares, cement and various other products.

Insoluble barium salts are non-toxic. Barium sulphate, for example, is used as a contrast material in the radiological diagnosis of ulcers. Soluble salts of barium, however, are readily absorbed through the skin or gastrointestinal tract and exhibit highly toxic effects on the nervous system if present in sufficient quantity. Symptoms include violent muscular spasms, paralysis and cardiac arrest. Trace quantities of soluble barium are known to accumulate in bones, but are not believed to exhibit a cumulative toxic effect like lead or cadmium. The maximum acceptable concentration of barium in a domestic water supply is 1.0 mg/l. There are currently no guidelines for livestock or irrigation waters. Levels of 1.0 mg/l are considered hazardous for marine organisms. The 96 hour LC 50 levels for invertebrates and fish are 10 -15 mg/l and 50 - 100 mg/l respectively. Barium can be concentrated from water by aquatic organisms by a factor of about 150.

BERYLLIUM

Beryllium is a trace element which does not usually occur in natural waters. It is present primarily as particulate rather than in dissolved form in all except very acidic conditions. Beryllium chloride, nitrate and sulphate are soluble in water but beryllium carbonate and hydroxide are insoluble. Levels seldom exceed 0.001 mg/l.

Weathering of minerals such as beryl accounts for some of the beryllium in natural waters but the majority comes from industrial discharge.

Beryllium metal has a high strength to weight ratio, a high melting point, high modulus of elasticity, high thermal conductivity and is quite resistant to corrosion. Beryllium copper alloys show many of the same properties. As a result, the metal and its alloys have found widespread use in the automotive, aircraft, spacecraft, missile and electrical industries. Beryllium is also used in X-ray lithography for reproduction of micro-miniature integrated circuits.

Beryllium and its salts are toxic and should be handled with the greatest of care. Exposure to beryllium dust should be limited to $2 \mu\text{g}/\text{m}^3$ over a 40 hour work week. The major toxic hazard potential comes from inhaling beryllium containing fumes and dusts. As yet, there are no drinking water guidelines.

At low levels, beryllium is toxic to plants, since it inhibits photosynthesis. Therefore irrigation waters used continuously should not contain concentrations greater than 0.10 mg/l. Levels of 0.011 mg/l may pose a threat to aquatic organisms in waters with a hardness of less than 75 mg/l of CaCO_3 .

CADMIUM

Cadmium levels in natural waters are generally below 0.01 mg/l unless there is an industrial source nearby. While weathering of rocks and minerals such as greenockite (cadmium sulphide) adds some cadmium to waters, the principal sources are industrial processes. Since greenockite is often found with zinc sulphide ore, the mining and smelting of zinc, lead and copper release large amounts of cadmium to the air and water. It may be discharged from the manufacturing of nickel cadmium batteries, various alloys, solders, ceramics, pigments and printing inks. It is also used in some pesticides and fertilizers. The burning of fossil fuels accounts for a large part of the cadmium emissions.

All forms of cadmium are highly toxic, and once ingested are likely to remain in the body for a long time, becoming concentrated in the liver, kidneys and other organs. Cases have been reported where toxic effects did not become apparent until many years after the last exposure. The water quality criteria for cadmium in domestic water supplies is 0.01 mg/l. The presence of cadmium reduces plant growth. Since it can be accumulated within plants which may be used as a food source, irrigation waters should not contain more than 0.050 mg/l when used on neutral or alkaline soils and 0.010 mg/l when used continuously on all soils. Levels above 0.0002 mg/l may be harmful to some aquatic organisms.

CALCIUM

Calcium ranks fifth in the order of natural elemental abundance and is commonly found in deposits of limestone, dolomite, gypsum and gypsiferous shale. It is generally present in all natural waters at levels which depend on the amount of contact with specific geological formations. Typically, levels in waters are less than 15 mg/l but waters in contact with carbonate rocks may contain up to 100 mg/l. For this reason, surface waters generally tend to have lower calcium levels than ground waters. The calcium content may vary from zero to several hundred milligrams per liter depending on the source and treatment of the water.

Calcium enters the waterway through leaching of soils, weathering of rocks, effluents of breweries and glue factories, and from road salt.

Calcium is an essential element for all life forms. It enhances biological productivity in plants and promotes proper bone and teeth formation in animals. Aqueous calcium compounds are generally non-toxic and represent no known health hazard.

While low concentrations of calcium assist in preventing the corrosion of metal pipes by forming a protective layer of calcium carbonate, high concentrations cause excessive scale which leads to restricted flow and pressure build-ups. Calcium is the major contributor to water "hardness".

A limit of 200 mg/l has been proposed for drinking water with an objective of 75 mg/l.

CHROMIUM

Chromium appears in nature primarily as the mineral chromite or chrome iron ore. Chromium in rocks and soils is generally present as insoluble chromic oxide. During weathering, chromium behaves like iron and is retained in sands and clays. Thus little of the chromium actually goes into solution. Natural waters generally contain less than 0.001 mg/l. Chromium(III) and chromium(VI) are the two forms most common in waters. The hexavalent form is the most common since above pH 5, the trivalent ion precipitates.

Chromium can enter the waterways as a result of weathering or from industrial discharges from cement, ferrochromium, chromium steel and metal plating industries. The burning of fossil fuels also contributes a large amount of chromium.

Chromium is present in small quantities in all soils and plants, but there is no evidence to indicate that it is required by plants for growth since chromic and chromate ions interfere with the uptake of essential elements. Soils with a high chromium content are generally infertile. Hexavalent chromium is the most toxic inorganic form of the element and has been demonstrated to exhibit carcinogenic properties. The maximum permissible concentration in domestic water supplies has been established at 0.05 mg/l of hexavalent chromium. Concentrations of chromium in unfiltered waters should not exceed 0.1 mg/l to protect aquatic life. The maximum allowable concentration of dusts and mists in air, measured as CrO_3 is 0.1 mg/m^3 per 8 hour exposure.

COBALT

Cobalt is found in nature in the minerals cobaltite, smaltite, and erythrite. Weathering of igneous and sedimentary rocks and soil leaching release cobalt to the water. Cobalt is also widely used in the manufacture of alloys and pigments used in glass staining. Recently it has found use as a binder in the tungsten carbide tool industry where it is commonly added to steel as a means of improving the cutting quality of certain tools. Cobalt is also used in the electroplating industry because of its appearance, hardness and resistance to oxidation. Levels in natural waters are generally lower than 0.0001 mg/l.

Cobalt is an essential element at trace levels for both animal and plant nutrition. It is known to be one of the main constituents of Vitamin B₁₂ and its presence is believed to be instrumental in the natural synthesis of this vitamin. Cobalt deficiency in man and animals results in a type of anemia which may be corrected by administering small dosages of cobalt chloride orally. Adverse effects due to cobalt are very slight even at high concentrations. Nausea and diarrhea are the common symptoms. No limits have been set on the maximum acceptable concentrations for cobalt in domestic water supplies. Tentative limits suggested by the Water Quality Criteria are 0.05 mg/l for continuous irrigation of all soils and 5 mg/l for use on fine textured soils. Cobalt is extremely toxic to Daphnia. The 48 hour LC 50 is 1.32 mg/l. Growth inhibition in carp has been observed above 5 mg/l.

COPPER

Copper occurs naturally as the free native metal as well as in malachite, cuprite, chalcopyrite and various mixtures of sulphide ores. The presence of copper in natural waters is generally due to industrial activity, but in certain circumstances, copper salts may be introduced to a water distribution system or reservoir as a means of controlling objectionable algae slimes. Copper may also be introduced into the environment through contact with brass and copper plumbing, discharges from industries making textiles, paints and electrical products, and discharges from smelting operations and tailings ponds.

Total copper in water may occur as insoluble particulates, soluble complexes and soluble divalent ions. Copper concentrations in the Great Lakes are dependent on local inputs, but in open waters they seldom exceed 5 µg/L.

Copper is an essential element to the human body, acting as a catalyst in many biochemical reactions. It is generally agreed that copper is an active agent in the synthesis of hemoglobin and that a copper deficiency in animals causes a severe anemia similar to iron deficiency anemia. A daily intake of about 2 mg is required for normal body functions. Excessive amounts of copper are toxic, and cause abdominal pain, vomiting and convulsions. Prolonged exposure results in liver and kidney damage. Copper poisoning occurs more often in grazing livestock than in man, usually resulting from consumption of dry feeds and grasses with high copper concentrations. The free aquated copper ion is extremely toxic to fish. When bound by organic ligands, the metal is biologically less active. Copper imparts a disagreeable taste to water at concentrations well below those which would be hazardous to health; hence the drinking water standard of 1.0 mg/l is based on taste rather than on toxicity. Levels in unfiltered samples should not exceed 0.005 mg/l to protect aquatic life.

IRON (TOTAL)

Iron is the most abundant of the heavy metals in nature, but is generally found at low levels in most surface waters because of the relatively low solubility of ferric iron when the pH exceeds 7. Compounds of iron in water are in a state of dynamic chemical equilibrium caused by bacterial activity and chemical oxidation. Under reducing conditions, iron exists in the ferrous state. On exposure to the atmosphere or oxidants, ferrous iron is oxidized to the ferric state and may hydrolyze to form insoluble ferric oxides. Because the relative concentrations of these compounds are constantly changing,

only a total iron determination provides relevant data. The most common forms of iron in water include ferrous ions, ferric ions, inorganic and organic chelates, complex colloidal organics, suspended oxides and hydroxides. Iron levels in surface waters are generally less than 0.5 mg/l but they often exceed this in groundwaters. Iron is added to the environment by weathering of iron bearing minerals such as pyrite, hematite and magnetite, mineral processing and acid mine drainage.

The role of iron is important in eutrophication studies as it affects retention and release mechanisms of phosphorus components in lake and river sediments. This ability of iron to bind up phosphorus is also important in nutrient removal programs at water pollution control plants where ferric chloride is sometimes used as a phosphate precipitant.

Iron is an important component of hemoglobin and an essential element for all life forms. It is non-toxic at high levels but is objectionable in domestic supplies because of the color and bitter taste it imparts. It is also known to cause reddish-brown stains on plumbing fixtures and laundry. The water quality objective for iron in domestic water supplies is 0.3 mg/l (filterable). Levels in an unfiltered sample should not exceed 0.3 mg/l to protect aquatic life.

LEAD

Lead is found in nature primarily as the mineral galena (lead sulphide) but also as cerussite, anglesite, crocoite, wulfenite, pyromorphite, matlockite and vanadate. Since the inorganic salts of lead are relatively insoluble, the element most commonly enters a water supply via industrial, mine and smelter discharges or through dissolution of old corroded lead plumbing. The latter problem is much more common in areas where the water is "soft"; that is, it has a low hardness or mineral content. Other sources of lead include the burning of leaded fuels and the production of lead batteries, explosives and paints. Levels in surface waters generally are below 0.04 mg/l.

Lead is an extremely toxic element which tends to exchange with calcium and accumulate in bone marrow. Lead toxicity may not appear until many years after the last exposure. Organic compounds of lead are many times more toxic than inorganic forms. The most common example is tetraethyl lead which is used as an "antiknock" additive in motor fuels. Organic lead exerts its toxic effect on the nervous system within a very short time causing mental confusion, delirium, nausea, hallucinations, insomnia, and convulsions. The maximum acceptable concentration of lead in domestic water is 0.05 mg/l. The toxicity of lead to aquatic life is highly dependent on the alkalinity. The toxicity generally decreases as the alkalinity increases. The total lead concentrations should not exceed the values below.

Alkalinity mg/l as CaCO ₃	Maximum Lead Concentration µg/L
< 20	5
20-40	10
40-80	20
> 80	25

The main sources of airborne lead pollution include internal combustion engines, industrial emissions from smelters, battery manufacturers and coal combustion. While the internal combustion engine is responsible for 95 - 98% of all lead emissions, some specific point sources cause abnormally high concentration of lead in air, soil and vegetation.

Lead and its compounds can be absorbed into the body through the respiratory and gastrointestinal tracts. Organic lead compounds can also be absorbed through the skin. Once ingested, lead is a cumulative poison. Symptoms of lead poisoning include fatigue, insomnia, mental confusion, delirium and eventual death. Lead has no known beneficial effects on plant or animal life.

Elevated levels of lead in the vicinity of lead smelting plants and busy highways has been a cause of concern. However, the effects of lead inhalation from the ambient air are not well known. Present Ontario guidelines for ambient air quality specify a maximum of 5 µg of lead per cubic meter of air, averaged over a 24 hour period.

LITHIUM

The element lithium is comparatively rare. It is concentrated in complex silicate minerals such as petalite and spodumene. Lithium is easily weathered and because lithium compounds are very soluble, they tend to stay in solution. It is rarely found in surface waters and water supplies, but it has been detected in hot springs and highly mineralized brines where the concentration is usually less than 10 mg/l. The major source of lithium in natural waters is industrial discharge.

Lithium is used in alloys, organic syntheses and special glasses. Lithium chloride and lithium stearate are used in de-humidifiers. Lithium hydride is very corrosive and irritating.

Lithium becomes toxic to animals and humans only if taken in very large amounts. When in sufficient quantity, it has the capacity for replacing sodium in body cells and thus upsetting intra-cellular metabolism. Cause of death is generally due to kidney failure although secondary effects on the nervous system are also observed. Small doses of lithium carbonate have been effectively used as an anti-depressant for treatment of psychiatric patients. No guidelines have yet to be set for drinking waters, but irrigation water must contain less than 2.5 mg/l.

MAGNESIUM

Magnesium ranks eighth in the natural order of elemental abundance and is commonly found in the minerals magnesite, dolomite, olivine, serpentine, and talc and asbestos. Its industrial applications include the manufacture of metal alloys, photographic flash bulbs, flares, cement, tanning solutions and explosives. It is also used in milk of magnesia and epsom salts.

Magnesium is present in all natural waters and is generally associated with the presence of calcium. In natural fresh water systems the calcium concentration is approximately three to five times that of magnesium. If the reverse is found, an artificial

source of magnesium is probable. Natural processes generally add more magnesium to the environment than do industrial processes.

Magnesium is an essential element for the life functions of all plants and animals. It is closely associated with the metabolic activity of phosphorus where it is believed to act as an activator of phosphate enzymes. Ingestion of magnesium produces no adverse effects at levels normally encountered in domestic water supplies, but concentrations in excess of 125 mg/l exert a cathartic action on the gastro-intestinal tract.

Magnesium is a major contributor to water "hardness"; therefore limitations on the amount of magnesium in water supplies are of concern especially to industry (see Hardness). Magnesium may also impart a disagreeable taste.

MANGANESE

Manganese is a common element in nature and found in numerous minerals as an oxide, sulphide, carbonate and a silicate. It is found at very low levels in natural waters, generally in the divalent (soluble) or quadrivalent (suspended) state, but in the absence of dissolved oxygen, the divalent state predominates. The permanganate anion, containing manganese in the heptavalent state, is not found in water unless it has been added artificially. High levels of manganese are usually the result of industrial discharges. Common industrial applications include the manufacture of metal alloys, dry cell batteries, paints, varnishes, inks, dyes, glass, ceramics, matches, fireworks, and fertilizer. Iron and steel plants and acid mine drainage account for a large portion of the manganese found in the environment.

Manganese in trace quantities is essential for the proper nutrition of both animals and plants and is believed to be important in the metabolic oxidation-reduction processes of living cells. Manganese deficiency in animals is characterized by lack of growth, bowing and abnormal fragility of the bones, and degeneration of the reproductive organs. Manganese and its salts, ingested in very large quantities, are potent nerve toxins.

Although manganese is non-toxic at the levels commonly encountered in water supplies, it causes unpleasant tastes, stains laundry and plumbing fixtures, and encourages the growth of objectionable micro-organisms at treatment plants. The maximum acceptable concentration of manganese for domestic water supplies in Ontario is 0.05 mg/l.

MOLYBDENUM

Molybdenum is found in nature as the minerals molybdenite, wulfenite, and molybdenum ochre. Its industrial applications include the manufacture of steel alloys, glass, ceramics, pigments, lacquers, paints, electrical wiring, and fertilizer. It is also used as a catalyst in certain chemical processes. Its presence in natural waters is likely the result of industrial discharges and agricultural run off as well as weathering processes.

In plants, molybdenum is believed to have an important influence on the ascorbic acid (vitamin C) equilibrium. It is essential to the nutrition of leguminous crops and all other nitrogen-fixing organisms. In animals, the function of molybdenum is not well

understood but it appears to behave much like copper. It is known to be a constituent of certain flavoprotein enzymes which have essential metabolic functions.

Of all the industrial heavy metals, molybdenum is one of the least toxic. Isolated instances of molybdenum poisoning are generally associated with cattle grazing in pastures where the soil contains elevated levels of molybdenum (20 - 100 mg/kg). Normal soils contain between 3 - 5 mg/kg. This condition in cattle is commonly known as "teart" and is characterized by severe diarrhea, anemia, and harsh discolored fur.

Molybdenum is rarely encountered in domestic water supplies and no limit has been specified for its maximum acceptable concentration. Levels should not exceed 0.010 mg/l for irrigation waters used continuously on all soil.

NICKEL

Nickel is more abundant in Ontario than anywhere else in the world. It is found primarily as the sulphide minerals pentlandite and pyrrhotite, as well as the arsenic mineral kupfernickel. Nickel has numerous industrial applications; the most important of which is the manufacture of high quality heat and corrosion resistant steel. Electroplating processes which utilize nickel are also widespread. Levels in natural waters are very low; therefore, if high nickel levels are found, they are likely the result of an industrial discharge or mine drainage. The median freshwater concentration of nickel in North American rivers is 0.10 µg/L.

Nickel and its salts have generally proven to be non-toxic to man even at very high levels. Nickel sulphate and nickel bromide have even been used therapeutically for headache, neuralgia, and insomnia. However, nickel carbonyl is suspected of being carcinogenic although confirmatory evidence is not well established. Contact with the metal can cause a severe form of dermatitis. The element appears to be biologically active, activating several enzyme systems, and appearing in high concentrations in ribonucleic acid. Toxicity to plants and invertebrates varies with the species. Acute nickel toxicity to fish is generally less than that of copper or zinc. The 48 hour LC_{50} for rainbow trout is 32 mg/l while it is 0.750 mg/l for copper and 4 mg/l for zinc. Concentrations of nickel in an unfiltered sample should not exceed 0.025 mg/l to protect aquatic life. Irrigation waters which are used continuously on all soil should not contain more than 0.20 mg/l.

POTASSIUM

Potassium ranks seventh in the natural order of elemental abundance. It is highly soluble and present at trace levels in all natural surface water, but very seldom exceeds 10 mg/l. Brackish or saline ground water, however, may contain potassium in excess of 100 mg/l. Potassium is generally found in the presence of sodium and only in rare instances will it ever exceed the sodium concentration. The sodium:potassium ratio in natural waters generally ranges from 2:1 to 3:1 with the proportion of sodium increasing with increasing concentrations. When potassium is in greater abundance than sodium, an artificial source is likely.

Potassium is an essential nutrient for all life forms, especially plants, and is therefore a major component of commercial fertilizers. Despite the substantial amounts of potassium dispersed over the land as fertilizer, it seldom appears in any great quantity in the water system. Many soils have the ability to fix potassium and prevent its migration through leaching. It is non-toxic and represents no known health hazard unless at extremely high levels. No limits have been specified for the maximum acceptable concentration of potassium in domestic water supplies.

SILVER

Silver is found in nature as the native metal, but its chief source is the mineral argentite. Besides being relatively rare, most of its compounds are insoluble; therefore it is highly unlikely that silver would appear in a domestic water supply unless introduced by artificial means. Weathering of argenite and lead-zinc ores containing silver may release silver to the environment. Freshwater concentrations seldom exceed 0.04 mg/l. It is commonly used in the manufacture of silver utensils, jewellery, and solders as well as in the electroplating, photographic, and food processing industries. Other sources include wastes from silver mines, industrial electronics plants and cloud seeding.

Exposure to silver in industry is usually manifested as a condition referred to as argyria; common symptoms include a permanent blue-gray discoloration of the skin and eyes. Although excessive exposure to silver may result in kidney, liver, or spleen damage, no recognizable disturbance of health need accompany this condition. Silver is accumulated in the skin indefinitely.

The maximum acceptable concentration of silver in domestic water supplies is 0.05 mg/l. This limit is used to restrict the use of silver compounds as disinfectants and is indirectly based on public health considerations. Colloidal elemental silver in water is highly toxic and may result in blindness or death. Concentrations of silver in an unfiltered sample should not exceed 0.1 µg/L to protect aquatic life.

SODIUM

Sodium is the sixth most abundant element on earth, comprising about 2.6% of the earth's crust, and is found in most natural waters. Weathering of salt deposits is the principal source of sodium in the aquatic environment. Concentration levels may vary from less than 1 mg/l to several thousand mg/l in some briny groundwaters. Sodium salts are used extensively in the manufacturing of chemical products as well as in commercial water softening devices which employ the sodium ion-exchange process. It is abundant in most industrial effluents. Waters that receive surface drainage from roads during the winter season can contain high levels of sodium if sodium chloride is used to control road ice.

Soils high in sodium are generally characterized by low infiltration and permeability rates and are therefore unsuitable for farming.

Sodium is an essential element for all life forms and is generally considered non-toxic. Patients with high blood pressure are often advised to consume water with less than 50 mg/l of sodium. Waters, softened by ion exchange processes may contain sodium in excess of this amount.

Since sodium is generally considered to be non-toxic, no drinking water criteria have been established.

STRONTIUM

Strontium occurs in nature as the minerals celestite and strontianite. It is not found as a native metal but it does exist in the environment as a sulphate or in combination with calcium and barium minerals. Natural compounds of strontium are relatively insoluble; therefore, its presence in domestic water supplies is generally very low. Strontium-88 is derived from weathering of strontium minerals and sedimentary rocks bearing sulphate and carbonate. The testing of nuclear bombs has caused fallout of the radioactive Strontium-90 isotope. Industrial applications of strontium and its compounds include the manufacture of metal alloys, storage batteries, paints, rubber, cathode ray tubes, tracer bullets and flares; its use, however, is not widespread. The radioactive isotope Sr-90 is one of the best Beta-emitters known, and thus provides a light-weight, long lived nuclear energy source.

The chemical behavior of strontium closely resembles that of calcium with a tendency to become concentrated in the human skeletal system. There are no known harmful effects from oral ingestion. It does not appear to be necessary for plant nutrition. There are no water quality guidelines set for non-radioactive strontium.

THALLIUM

Thallium occurs in the minerals crooksite, lorandite and hutchinsonite. It is also present in pyrites. Generally, the only commercial sources of thallium are flue dusts and residues from metallurgical and chemical processes such as smelting of lead and zinc ores in which thallium is a minor impurity. Thallium is generally present in only trace amounts in surface waters. The monovalent ion is the most stable form in aqueous solution.

Thallium readily alloys with a number of metals to form a number of low melting binary and higher order alloys. It has some applications in the electronics and glass industries. Thallium sulphate was an important ingredient in rodenticides and pesticides, but its toxicity to humans has curtailed this use.

The element and its compounds are toxic and should be handled carefully. Contact of the metal with skin is dangerous and may be carcinogenic to

humans. Thallium has been used to treat ringworm and other skin infections but its use is limited because the margin of safety between therapeutic and toxic doses is too narrow. Thallium can inhibit photosynthetic processes and plant transpiration. In both fish and invertebrates it acts as a neuro-poison. The 96 hour LC 50 for rainbow trout is less than 10 mg/l. No drinking or livestock water guidelines have been set in Ontario. The U.S.A. EPA has defined an ambient water quality criteria of 4 $\mu\text{g/L}$.

TIN

Tin is found mainly in the mineral cassiterite. Most of the world's supply comes from Malaysia, Bolivia, Indonesia, Nigeria and Thailand. Tin is derived naturally from weathering processes. Tin is readily adsorbed by clay minerals so it is generally only found in trace amounts. The major sources of tin in the environment are industrial discharges.

Tin foil is used for electrical condensers, bottle cap liners and food wrappings. Tin wire is used for fuses and safety plugs and is used in the production of plate glass. Tin plating over steel is the most important use of tin. Tin cans in the food packaging are the major products. Tin alloys may be used in superconductive magnets. Tin also has a number of organic applications.

Tin and its inorganic compounds are essentially non-toxic. The amounts tolerated by the human body are far higher than would normally be ingested by eating canned foods. Many organic tin compounds are toxic, and may in addition be irritating to skin, mucous membrane and other tissues. Diethyl tin can cause severe lesions and even death. There are no guidelines for inorganic tin in drinking water. Since tin is not generally taken up by plants there are no agricultural guidelines.

TITANIUM

Titanium is found in nature as the minerals rutile, ilmenite, and sphene. It ranks eighth in the natural abundance of elements and is readily found in most soils, varying between 0.5% and 10%. The high tensile strength and corrosion resistant properties of titanium metal are valuable in the manufacture of special steel alloys which are used for structural purposes in aircraft frames and engines. Titanium metal is also used in the manufacture of electrodes, lamp filaments, and X-ray tubes. Oxides of titanium are used as a white pigment in the paint, glass, and ceramics industries. Titanium and its salts are generally extremely inert and insoluble; the notable exception is titanium tetrachloride, which is highly corrosive.

Neither plants nor animals accumulate titanium to any extent. There is no evidence that titanium plays an essential role in human or animal nutrition.

There is no known toxicity to humans, animals, or plants. Surgical appliances are generally made of titanium and various titanium derivatives

have been used in the treatment of skin disorders. No limits have been specified for the maximum acceptable concentration of titanium in domestic water supplies.

VANADIUM

Vanadium occurs in nature as the minerals vanadinite, patronite, mottramite, and carnotite, and is generally found in the presence of selenium. Metallic vanadium does not occur in nature. Trivalent vanadium salts tend to be insoluble but tetra- and pentavalent salts tend to be soluble. Seepage from carbonaceous deposits may be a major source of vanadium. Other sources include weathering and industrial discharges. Vanadium is used in the steel industry where it is known to increase the general toughness and tensile strength of certain alloys, but it is also used in the manufacture of dyes, paints, inks and ceramics. Vanadium has been found in all fuel and motor oils and has been used for oil spill identification by means of the nickel-vanadium ratio. Vanadium in surface waters is generally found at levels below 0.05 mg/l.

The usefulness of vanadium in animal and plant metabolism has not been convincingly demonstrated and its behaviour in biological systems is not well understood. Some experimental evidence suggests that the presence of vanadium in trace quantities may be beneficial in the prevention of heart disease.

Several compounds of vanadium are known to be toxic in high concentration. Exposure may produce nausea, diarrhea, abdominal pain, nervous trembling of the hands, and greenish-black discoloration of the tongue. No limits have been specified for the maximum concentration of vanadium in domestic water supplies because the element is rarely encountered. Levels in livestock drinking waters should not exceed 0.1 mg/l.

ZINC

Zinc is commonly found in nature as zinc sulphide in the mineral sphalerite and as zinc carbonate in the mineral smithsonite. It is widely used in the manufacture of metal alloys such as brass, batteries, dyestuffs, paints, ointments and rubber, as well as in electroplating. Zinc oxide is used in medical ointments and as a pigment. Zinc carbonate and sulphide are used as white pigments in paints and enamels. The sulphide is also used as a phosphor for television screens and fluorescent coatings. Zinc most commonly enters a water supply via the deterioration of galvanized iron, the dezincification of brass, direct industrial discharges, and drainage from mine wastes. It is usually found in concentrations less than 0.05 mg/l in most natural waters but acidic waters may hold up to 50 mg/l.

Zinc is essential to plant and animal life. It is a component of several metalloenzymes and proteins. Deficiencies result in growth retardation, skin lesions, bone weaknesses, poor healing of wounds, impaired reproductive processes and reduced learning capacity.

Plants growing in a zinc deficient soil are severely stunted. In animals, zinc is an important constituent of a number of enzymes, notably carbonic anhydrase which is vital for normal respiration. Zinc and its compounds are relatively non-toxic when taken orally. Even at high concentrations, nausea and diarrhea are the only adverse effects likely to be encountered. The limiting factors which determine the acceptable maximum concentration in a water supply are taste and appearance. Zinc, in excess of 5 mg/l, imparts a bitter astringent taste when the water is boiled and may give a milky appearance in alkaline waters. The maximum acceptable limit for domestic water supplies in Ontario is therefore 5 mg/l. Concentrations of zinc in an unfiltered sample should not exceed 0.030 mg/l to protect aquatic life. Zinc toxicity increases with increasing temperature and decreasing dissolved oxygen. The presence of copper and cadmium enhance the sublethal toxicity of zinc.

Sample Handling and Preservation

Surface Waters, Drinking Waters, Precipitation

If necessary, a 25 ml sample is sufficient for the determination of most elements. Routine procedures require a 500 ml sample obtained in an acid washed plastic bottle. Samples are preserved by the addition of approximately 10 drops of nitric acid per 500 ml sample (pH \approx 2). If a sample is suspected of having high alkalinity additional nitric acid should be added.

NOTE: Because of the extremely low levels determined, bottles and acid must be obtained from the laboratory to ensure an uncontaminated sample.

Sewage and Industrial Wastes

100 ml is sufficient but 500 ml in a glass bottle acid preserved as above is preferred.

NOTE: Foil cap liners if present must be removed to prevent sample contamination. If low level determinations are required on relatively "clean" effluents they should be treated as surface waters and sampled accordingly.

Sewage Sludge

10 ml is adequate but 100 ml in a wide mouth jar no more than $\frac{1}{2}$ full is requested. Acid preservation is not essential but is preferred.

NOTE: Full containers in narrow mouth bottles can explode.

Soil and Sediment

Care should be taken when sampling to ensure that samples are representative of the area being examined. Sediment samples should be transported directly to the laboratory in glass jars with nonmetallic caps. Sediments from different sampling points should be kept separate to avoid cross-contamination. Sampling devices and containers should be designed so as to minimize the chances of metal contamination. Sediments are not dried.

Soil samples are normally collected in conjunction with vegetation samples as an aid to the differentiation between current and past emission situations. Occasionally, soil samples are collected to establish background conditions.

Soil is collected with a 2 cm ($\frac{3}{4}$ ") diameter stainless steel tube. A minimum of 10 cores are taken from the sampling site. All soil samples are collected in triplicate (i.e. minimum 3 x 10 cores) and the collection form is completed comprehensively describing the texture of the soil and the overall sampling site. Each core must be separated into fractional depths of 0 - 5 cm, 5 - 10 cm and 10 - 15 cm, and each level is placed in an appropriately labelled plastic bag or glass jar with a nonmetallic cap for shipping. Upon arrival at the laboratory, samples are generally air dried.

Ideally, soils should be sampled from an entirely undisturbed or sodded area. Contaminated situations should be matched as closely as possible with conditions existing immediately outside the area.

Vegetation

To ensure correct interpretation of analytical data, the plant species, the age or maturity of leaf tissues, the age of tree or shrub, and the position of sample on the tree or shrub should be recorded for each sample. Usually, foliage is collected from the side of the tree or shrub facing the presumed source of air pollution but, occasionally, a second sample may be taken from the side opposite the source. Samples are taken by trimming outside growth from ground level up to 7 meters or more and collecting all leaves to provide a composite sample of 500 to 1,000 grams of fresh material.

Current practice dictates the collection of 3 samples from each sampling location (triplicate sampling). Samples are placed in perforated polyethylene bags and transferred to refrigerated storage as soon as possible for processing by the Phytotoxicology Laboratory. Forage samples (grass) are collected by cutting the terminal 25 cm (10") of stems and blades over the representative area to be sampled, at 10 step intervals. Dried flower heads and stalks are discarded and no root material whatsoever is included. The different forage species included in the sample are identified and are representative of the population of the species in the field.

Any sample contaminated by roadside dust should be noted in the accompanying request form. All vegetation samples, as collected are potentially unstable, and will decompose unless properly handled. Vegetation samples can be preserved for a few weeks under refrigeration; when dried at 80°C for 30 hours in a forced draft oven, they become almost permanently stable.

Biological Material (Fish)

Subsequent to weighing, the fish is placed on a wooden board covered with paper towelling. An incision is made with a stainless steel knife on the dorsal surface posterior to the nape, and the body is cut ventrally to a point below and posterior to the pectoral fin. The sex of the fish is noted and recorded.

The fish is cut posteriorly along the dorsal surface. The epaxial musculature (muscle above the lateral line) is stripped from the skin and about 100 g is placed in a plastic "whirlbag" and frozen immediately to retain moisture and retard protein breakdown.

Dust Fall

The dustfall jar is covered and returned to the laboratory as is. There the plastic bag insert containing the sample is removed and the container is returned to the field.

Hi-Vol Filters

See The Determination of Suspended Air Particulate.

Lo-Vol Filters, Andersen Filters

The same principles used for Hi-Vol filters are applied except that because of the much lower metal levels extra care is taken to prevent contamination through handling. Disposable plastic gloves are worn during all operations. Exposed filters are folded and placed in appropriately sized plastic "whirlbags".

Selection of Method

Atomic spectroscopy in its various forms has replaced colorimetry as the method of choice for most metal analysis. Speed, superior detection limits and relative freedom from interferences all favor these techniques. Certain applications are best done by Anodic Stripping Voltammetry. At this time, over 90% of all trace metals analyses are accomplished by atomic spectroscopy and the majority shall be in the foreseeable future.

Atomic spectroscopy is used as a collective term to describe five analytical techniques:

- flame atomic absorption spectrometry
- flameless atomic absorption spectrometry
- inductively coupled plasma atomic emission spectroscopy
- d.c. arc atomic emission spectrography
- X-ray fluorescence spectroscopy

The first three are used to analyze solutions, usually acid digests, while the fourth and fifth are applied directly to solid samples.

The analytical process consists of two main steps, sample preparation and analytical finish.

The purpose of sample preparation is to solubilize the elements of interest while removing most organics by oxidation and volatilization. Care must be taken to avoid the formation of insoluble oxides or conversely volatile halides for certain elements. Each sample matrix is subjected to different acid attack in accord with the above requirements. The acid digests are suitable for atomic absorption (AA) or atomic emission (ICP) analysis. It is known that most acid digestion procedures do not completely solubilize certain elements, for example, aluminum in silicates. If true totals are required, it is possible to use more extensive digestion procedures, or preferably, to use DC arc or XRF procedures which are not subject to the vagaries of acid digestion.

The choice of analytical finish, ICP, or flame or flameless AAS is the result of parameter list, sample volume, required detection limits, throughput, etc. If correctly applied, the techniques produce equivalent, unbiased data.

The selection criteria are discussed below.

D.C. Arc Atomic Emission (DC Arc)

Because of the photographic nature of the technique it is ideal for preliminary identification of unknowns and samples from previously untested areas. The levels of over forty elements can be determined and a permanent photographic record is retained which can be checked for other elements if the need arises.

The procedure also provides true "total" metals data as it completely volatilizes solid samples providing accurate levels for elements such as tin, silica, and aluminum which can be particularly resistant to routine acid digestion procedures. The technique is, however, relatively tedious and standard preparation is difficult so that it is not the method of choice for high volume defined programs, e.g. a lake survey for Pb, Cd, Zn, Cu.

X-Ray Fluorescence (XRF)

With this procedure, solid samples are analyzed directly by bombardment with X-rays and the detection and quantitation of the fluorescent X-rays produced. X-ray fluorescence (XRF) is best applied to the analysis of smooth, homogeneous surfaces and is thus ideal for the analysis of air particulates deposited on membrane filters. It is also applied to the analysis of major elements in vegetation, S, Cl, K, P, Ca, after the sample is compressed with paraffin into a smooth pellet. XRF is normally applicable to all elements heavier than Mg in the periodic table; however, detection limits and interferences vary. The lab has recently obtained a new instrument capable of producing simultaneous analyses on twenty elements.

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP)

The procedure is capable of analyzing solutions simultaneously for from 10 to 24 elements dependent on the matrix. Standards should be matrix matched and the major matrix elements relatively consistent. Thus, the procedure is ideal for surveys requiring several elements on a single sample type. For example, Zn, Cu, Ni, Pb, Cd, Cr, Co, As, Se on sewage sludge, or Zn, Cu, Pb, Ni, Co, Mn, Fe, Al on lake waters and tributaries. Detection limits are equivalent or superior to flame AAS for most elements. The standardization process and actual time for sample analysis takes significantly longer than single atomic absorption determinations. ICP is usually the method of choice for samples requiring 4 or more elements.

Flame Atomic Absorption (AAS)

This is the most widely applied analytical atomic spectroscopy procedure. After set up and standardization, a single analysis consumes less than one ml of sample, takes only a few seconds and is relatively interference free. Modern instruments have highly automated setup procedures and can readily be interfaced to minicomputers for unattended data collection and manipulation. When correctly applied, over a wide range of elements, matrices and concentrations ICP and flame AAS produce equivalent, unbiased data. For parameters determinable by either procedure, the final choice of technique depends primarily on the number of elements requested. Automated flame AAS is faster for samples requiring the determination of 1 to 4 elements and is therefore the method choice in these cases. Manual flame AAS is used primarily for less commonly requested elements such as lithium and silver.

Flameless Atomic Absorption Spectroscopy (FAAS)

The procedure is equivalent to flame AAS except that a resistively heated graphite tube is used as the atomizing device instead of a flame. Flameless AAS is inherently 10 to 50 times more sensitive than flame AAS and requires only 20 μ l per determination. A single analysis requires one minute as compared to 5 seconds for flame AAS. The other disadvantage is a tendency to chemical matrix interferences. Studies have shown that 20x preconcentration flame AAS provides somewhat better precision and accuracy than flameless AAS. Thus for samples requiring low detection limits, such as natural and potable waters where sufficient sample is obtainable, preconcentration - flame AAS is the preferred procedure. Where this is not the case, as for precipitation event samples or low volume filters, graphite furnace AAS is used. For difficult determinations such as lead in whole fish, where low levels are combined with high salt content, graphite furnace with standard additions is employed.

The following table (Table 0.1) lists the elements determined by each procedure, both on a routine and non-routine basis. Also, the currently employed analytical schemes are listed.

It is important to emphasize currently. At this point methodologies are rapidly being refined, amended and altered. The main motivational force is internal laboratory logistics to meet increasing sample loads and new program requirements. AAS and ICP data are unbiased and thus the procedures are used interchangeably as indicated above.

TABLE 0.1
ROUTINE SAMPLE ROUTING

Matrix	Preparation	Technique	Elements
1. Rivers, Lakes and Drinking Waters	Preconc. 5.1.2	ICP or AAS	Be, Mg, Al, Ca, Ti V, Cr, Mn, Fe, Co Ni, Cu, Zn, Mo, Cd Ba, Pb
2. Acid Leaches/ Throughfalls	None 5.1.1	FAAS	Al, Cd, Cu, Fe Mn, Ni, Pb, V
3. Aqueous Industrial Wastes	Nitric Evap. 5.1.3 Aqua regia dign 5.1.4	AA	as for 1
4. Sediments	Aqua regia 5.2.1-3	ICP	Cu, Ni, Zn, Cd, Co Cr, Pb, Fe, Mn, Al Ca, Mg, V, Mo, Ba Sn, Be, Ti
5. Sewage	Aqua regia 5.3.1	ICP AAS backup	Cu, Zn, Ni, Pb, Cd Cr, Mn, Fe, Al req. for Cu, Ni, Cd Co, Pb, Ba, Mo if Fe > 1000 mg/l
6. Vegetation	Aqua regia 5.4.1 HClO ₄ .HNO ₃ 5.4.2 Pelletization 5.3	ICP AAS XRF	Cu, Zn, Ni, Pb, Cd Cr, Mn, Fe, Al, Ca Mg, V, Mo, Ba, B as above except B Ca, K, Cl, S, P, Si
7. Soils	HClO ₄ .HNO ₃ 5.5.1	AAS	as for 6. AAS
8. Biomaterials	HClO ₄ .HNO ₃ 5.6	ICP or AAS	Mg, Al, Ca, Cr, Mn Fe, Co, Ni, Ca, Zn Cd, Pb
9. Air particulates (glass fibre filters)	HF 5.7.1 HNO ₃ 5.7.2	ICP or AAS AAS	Cd, Co, Cr, Cu, Fe Mn, Ni, Pb, V, Zn Pb
10. Other filter media	None, Method 5-1 Aqua, regia 5.7.3 None, Method 5-2	XRF ICP or AAS XRF	Pb as for 9. Ca, Pb
11. Dustfall	Aqua regia 5.10	AAS	as for 9.

THE DETERMINATION OF TRACE METALS By Atomic Spectroscopy

Method 1

Flame Atomic Absorption Spectrophotometry (AAS)

SUMMARY

Matrix:	Surface waters, drinking waters, sewage, sludges, soils, sediments, vegetation, air particulates and biomaterials.
Substance determined.	Li, Be, Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Mo, Ag, Cd, Ba, Au, Tl, Pb.
Interpretation of results.	Results are reported as the element in mg/l, mg/kg or $\mu\text{g}/\text{m}^3$.
Principle of method.	An aliquot of sample is prepared by wet acid digestion to render it suitable for aspiration into a flame. The metal ions are reduced to free atoms by the reducing zone of the flame. Atoms in the elemental state absorb energy of very specific wavelengths which are different for each element. An atom of aluminum, for example, will absorb energy provided by a hollow cathode lamp which emits the elemental spectrum of aluminum. The energy absorbed is directly proportional to the concentration of atoms in the original system.
Time required for analysis.	A single digestion requires about half a day. About 80 can be done at one time in batches. An individual AAS analysis for a single element requires only a few seconds. However, changing lamps, machine set up, organizing and reporting requires considerable time.
Range of application.	See Table 1.1.
Standard deviation.	See Table 1.1.
Accuracy.	See Table 1.1.
Detection criteria.	See Table 1.1
Interferences and shortcomings.	AAS techniques are relatively free of interferences, however stable compound information, incomplete volatilization, matrix effects, scatter and background absorption can all occur. These effects can be suppressed by the addition of certain reagents or by background correction using a hydrogen or deuterium arc.

**Mimimum volume
of sample.**

See Table 1.2.

**Preservation and
sample container.**

See Table 1.2.

**Safety
considerations.**

Extreme care should be taken in the handling and use of the various concentrated acids especially aqua regia and perchloric acid. Digestions should be carried out in a well ventilated fumehood. Wear eye protection at all times when digesting samples or handling acids. The atomic absorption unit should have a proper exhaust canopy to expel the heat and fumes. Ensure that the gas cylinders are properly secured. Ensure that the proper instructions for the operation of the atomic absorption unit are followed especially ensuring that the waste trap is filled with water at all times and the fuel is shut off first.

TABLE 1.1

AUTOMATED ATOMIC ABSORPTION
(all values are in mg/l)

PE 5000

Digestate**	Element	Detection Limit	Top of Range	Precision Between Run	Accuracy	Notes
"Preconc."	Al	.04	20	.98 ± .09	-.02 at 1	2
Aqueous Acid	Cd	.004	5	.19 ± .01	-.01 at .2	1
Digest	Co	.04	5	.24 ± .01	-.01 at .25	1
≈ 5% HNO ₃	Cr	.04	5	.24 ± .01	-.01 at .25	2
+ .1% K	Cu	.02	5	.19 ± .01	-.01 at .2	
	Fe	.05	20	.99 ± .08	-.01 at 1	2
	Mn	.04	5	.19 ± .01	-.01 at .2	
	Ni	.04	5	.47 ± .02	-.03 at .5	1
	Pb	.06	5	.24 ± .01	-.01 at .25	1
	Zn	.02	5	.19 ± .00	-.01 at .2	1
"Glass Fibre"	Cd	.004	1	.013 ± .004		1
Aqueous	Co	.04	2	-		1
5% HNO ₃	Cr	.04	2	.30 ± .05		2
+ Radiation	Cu	.02	5	6.4 ± .8		
	Fe	.05	50	19.5 ± 1.1		2
	Mn	.04	5	.79 ± .08		
	Mo	.04	1	-		2
	Ni	.04	5	.28 ± .06		1
	Pb	.06	20	3.8 ± .12		1
	V	.1	2	.12 ± .03		2
	Zn	.02	20	9.3 ± .8		1
Sediment	Ag	.02	10			1
5% HNO ₃	Al	.04	50	4.7 ± .3	+.01 at .70	2
.1% K	Au	.04	20			1
	Ba	.04	10	2.9 ± .2		2
	Be	.02	5			
	Co	.04	5	.053 ± .004	-.02 at .50	1
	Cu	.02	5	3.8 ± .1	-.01 at .40	
	Fe	.05	50	4.40 ± .14	-.01 at .60	2
	Li	.02	5			
	Mn	.02	5	1.9 ± .06	-.01 at .35	
	Mo	.04	5	.14 ± .01		2
	Ni	.04	5	.54 ± .04	-.02 at 1.0	1
	Pb	.06	5	6.4 ± .2	-.02 at .50	1
	Sr	.04	5	.4 ± .01		
	Ti	.2	50			2
	V	.2	20	.98 ± .05	-.01 at .75	2
	Zn	.02	5	11.4 ± .3	-.01 at .40	1

TABLE 1.1 (con't.)

MANUAL ATOMIC ABSORPTION
(all values are in mg/l)

PE 503, Varian AA 1250, Varian AA6, PE 403

Digestate	Element	Detection Limit	Top of Range	Precision * Between Run	Accuracy	Notes
Water or Sediment						
5% HNO ₃ or dilute	Ag	.01	2	.19 ± .01	-.01 at .20	1
	Al	.2	50	*		
HCl-HNO ₃	Au	.04	20	NA		1
+ .1% K	Ba	.04	10	.20 ± .02	.00 at .20	2
	Be	.02	5	*		
	Cd	.006	2	.87 ± .03	-.03 at .40	1
	Co	.02	5	*		
	Cu	.02	5	*		
	Fe	.2	50	*		
	Li	.01	1	.09 ± .006	-.01 at .20	
	Mn	.02	5			
	Mo	.04	5	.20 ± .02	.00 at .20	2
	Ni	.06	5	*		
	Pb	.06	20	*		
	Sn	.8	50	NA		
	Sr	.04	5	.21 ± .02	+.01 at .20	
	Ti	.2	5	NA		2
	Tl	.04	20	NA		1
	V	.04	5	.21 ± .02	+.01 at .20	2
	Zn	.02	2	*		
dilute digestate with .5% La	Ca	.03	3	23.7 ± 1.6	-1.3 at 25.	
	Mg	.01	1	10.0 ± .69	0.0 at 10.	
dilute digestate with .1% Cs	K	.02	2	40.2 ± 2.4	-.3 at 7.2	
	Na	.02	2	10.1 ± .9	0.0 at 40	

* - Normally analyzed on PE5000

NA - Not available - insufficient data

** - For details see section 5, in this method.

All analyses are performed with an Air/Acetylene flame without background correction unless otherwise noted.

Note 1: Background correction

Note 2: Nitrous Oxide/Acetylene flame.

TABLE 1.2
SAMPLING AND SAMPLE HANDLING

Matrix	Container	Sample Size		Preservatives
		Minimum	Optimum	
Surface & Drinking Water	Acid washed plastic bottle	100 ml	500 ml	20 drops HNO ₃ pH =< 2
Surface & Drinking wastes and, effluents	Glass bottle	100 ml	500 ml	20 drops HNO ₃ pH =< 2
Sediments	Glass or plastic jar	2 g	25 g	none
Sewage	Glass Bottle ½ full	10	500 ml	20 drops HNO ₃
Vegetation	"Whirlbag"	2 g	25 g	none
Soils	as for sediments			
Biomaterials	"Whirlbag"	5 g	25 g	none
Air particulates	Envelope	50 cm ²	500 cm ²	none

THE DETERMINATION OF TRACE METALS by Atomic Spectroscopy

METHOD 1

Flame Atomic Absorption Spectrophotometry Method 1

1. Introduction

Atomic absorption spectrophotometry is an analytical technique for the determination of elements based on the absorption of radiation by free atoms. The production of atoms from a chemical compound requires the absorption of energy. The energy is usually supplied in the form of heat from a flame. The absorption of thermal energy from the flame, with a subsequent re-emission of the energy as a spectral line, corresponds to atomic emission. Atomic absorption corresponds to the absorption of energy from a source other than the flame (usually the signal from a hollow cathode lamp) with the subsequent decrease in the signal from the source. To determine total or leachable elements, the sample aliquot is either digested, leached or fused (after dry ignition). This preparation breaks down any organic matter, solubilizes any particulate matter and if the matter cannot be dissolved, stabilizes it prior to filtration. After the proper preparation of sample, the resultant solution should be clear and colorless (as far as possible).

2. Interferences and Shortcomings

As was indicated previously, the digestion process may volatilize or incompletely solubilize analytes of interest, producing low results.

Because of the simplicity of absorption spectra, there are very few known examples of actual spectral interferences. Interferences which actually influence the proportion of atoms in the flame available to absorb the resonance radiation arise largely from the chemical effects which originate in the flame itself, or in the sample solution. Such interferences are usually caused by the formation of stable compounds or by ionization.

In addition, minor interferences are caused by various physical phenomena, including incomplete volatilization of the solid particles formed in the flame, variations in the physical properties of solutions (matrix effects), scatter and background absorption.

2.1. Chemical Interferences

2.1.1. Stable Compound Formation

This arises because compounds or radicals containing the element being measured are not broken down into individual atoms at the temperature of the flame being used. Stable compounds can even be formed in the flame. Examples of this are:

2.1.1.1. The lowering of alkaline earth metal absorbances in the presence of large excesses of aluminate, silicate, phosphate and some other oxy-anions.

A releasing agent such as lanthanum chloride is added which forms stable oxy-salt complexes thus freeing the alkaline earth ions and facilitating their reduction.

- 2.1.1.2. Metals which form refractory oxides (Al, V, Si, Ti) have low sensitivity.

A combination of the high temperature reducing $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame and the addition of large excesses of elements which also form stable oxides and thus compete for the available oxygen in the flame, increases sensitivity.

- 2.1.1.3. The calcium signal is depressed in the presence of protein. This is compensated for by a thorough sample digestion.

2.1.2. Ionization

Many metals, including aluminum, silicon and chromium, are ionized to an appreciable extent at the hot flame temperatures of nitrous oxide-acetylene. A potassium or lithium concentration of approximately 1000 mg/l will usually minimize this ionization effect.

2.2. Physical Interferences

2.2.1. Incomplete Volatilization

This implies that, at the flame temperature used, the droplets produced by the nebulizer have given rise to solid particles which, because of their vaporization temperature or speed through the flame, or both, are not completely converted to a vapour. This type of interference is usually caused by the formation of metal-metal solutions of high boiling point under reducing conditions. An example of this is the depression of the chromium signal in the presence of high iron concentrations in an air-acetylene flame. This type of interference is usually non-existent in a nitrous oxide-acetylene flame.

2.2.2. Matrix Effects

These influence the number of atoms actually entering the flame, and arise from differences in physical properties of the sample. This can be caused by varying the acid concentrations which in turn can cause a difference in viscosity or surface tension. In order to help compensate for this effect, samples, standards, and blanks should be made up to the same acid or salt concentration.

2.2.3. Scatter and Background Absorption

This is the result of either small solid particles in the flame which scatter some of the hollow cathode radiation, or discrete molecules which absorb some incident energy.

The effect is compensated for by use of deuterium background correction or use of a non-absorbing line. Specific examples are: (1) molecular absorption on the lead ($2,320\text{\AA}$) line by calcium, magnes-

ium, sodium and potassium, (2) molecular absorption on the nickel ($2,320\text{\AA}$) line by calcium, (3) molecular absorption on the cadmium ($2,288\text{\AA}$) line by sodium chloride.

3. Apparatus

- 3.1. Atomic Absorption Spectrophotometers, Varian Techtron AA1250 and AA6, Perkin Elmer, 603 and 5000 equipped with direct concentration readout recorders and/or interfaced to microcomputers.
- 3.2. Hot plates. Large Lindberg or Thermoline. Use only in a well ventilated fume hood.
- 3.3. Folin digestion tubes, graduated at 25 and 50 ml.
- 3.4. Folin digestion tubes, graduated at 100, 10 and 5 ml.
- 3.5. 250 ml graduated Griffin beakers.
- 3.6. Speedivap beaker covers for 250 ml beakers.
- 3.7. Glassware: Measuring cylinders.
Various pipets (volumetric).
Regular laboratory glassware.
- 3.8. Millipore filtration unit (glass) equipped with filters ($0.45\text{ }\mu\text{m}$ size).
- 3.9. Acid dispensers (Oxford pipettors).
- 3.10. Thermolyne muffle furnace type 1300.
- 3.11. Graphite crucibles.
- 3.12. Crucible tongs.
- 3.13. Magnetic stirrer with stirring bars.
- 3.14. Drying ovens, capable of 96°C .
- 3.15. Technicon hot blocks, model BD40
- 3.16. Eppendorf micro pipettes
- 3.17. Miele Dishwashers, with appropriate washing programs.
- 3.18. Corning Megapure still.

4. Reagents

- 4.1. Sulphuric acid, (H_2SO_4) concentrated, "Aristar" (high purity grade-low in metals) or Baker "Ultrex".
- 4.2. Nitric acid, (HNO_3) concentrated, reagent grade.

4.3. Hydrochloric acid, (HCl) concentrated, reagent grade.

4.4. Perchloric acid, (HClO₄) concentrated, reagent grade.

4.5. Anti-bumping granules.

4.6. Glass beads.

4.7. Stock Lithium Solution

Dissolve 5.324 g lithium carbonate in the minimum volume of 1:5 HNO₃ and dilute to 1,000 ml with distilled water.

1 ml = 1,000 µg Li.

4.8. Stock Beryllium Solution

Dissolve 1.000 g of beryllium metal in a minimum volume of 6M HCl. Dilute to 1,000 ml with 1% HCl.

1 ml = 1,000 µg Be.

4.9. Stock Sodium Solution

Dissolve 2.542 g of sodium chloride in distilled water and dilute to one liter.

1 ml = 1,000 µg Na.

4.10. Stock Magnesium Solution

Dissolve 1.000 g magnesium ribbon in concentrated hydrochloric acid. Dilute to 1,000 ml with distilled water.

1 ml = 1,000 µg Mg.

4.11. Stock Aluminum Solution

Dissolve 1.000 g aluminum wire in nitric acid. Dilute to 1,000 ml with distilled water.

1 ml = 1,000 µg Al.

4.12. Stock Potassium Solution

Dissolve 1.9076 g potassium chloride in distilled water and dilute to 1 liter.

1 ml = 1,000 µg K.

4.13. Stock Calcium Solution

Dissolve 2.497 g of calcium carbonate in concentrated hydrochloric acid. Dilute to 1,000 ml with distilled water.

1 ml = 1,000 µg Ca.

4.14. Stock Titanium Solution

Dissolve 1.000 g of titanium metal in 1:1 HCl with heating. Cool and dilute to 1,000 ml with 1:1 HCl.

1 ml = 1,000 µg Ti.

4.15. Stock Vanadium Solution

Dissolve 1.000 g of vanadium metal in a minimum volume of HNO₃ and dilute to 1,000 ml with 1% HNO₃.

1 ml = 1,000 µg V.

4.16. Stock Chromium Solution

Dissolve 1,000 g of chromium metal in 1:1 hydrochloric acid with gentle heating. Cool and dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Cr.

4.17. Stock Manganese Solution

Dissolve 1,000 g manganese metal in the minimum volume of 1:1 nitric acid and dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Mn.

4.18. Stock Iron Solution

Dissolve 1,000 g of iron wire in concentrated hydrochloric acid. Dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Fe.

4.19. Stock Cobalt Solution

Dissolve 1,000 g cobalt in 1:1 nitric acid and dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Co.

4.20. Stock Nickel Solution

Dissolve 1,000 g nickel metal in aqua regia. Dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Ni.

4.21. Stock Copper Solution

Dissolve 1,000 g of copper wire in concentrated hydrochloric acid. Dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Cu.

4.22. Stock Zinc Solution

Dissolve 1,000 g of zinc powder in concentrated hydrochloric acid. Dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Zn.

4.23. Stock Strontium Solution

Dissolve 1.685 g strontium carbonate in 10 ml 1:1 nitric acid and dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Sr.

4.24. Stock Molybdenum Solution

Dissolve 1,000 g molybdenum metal in concentrated HNO_3 with gentle heating. Cool and dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Mo.

4.25. Stock Silver Solution

Dissolve 1,000 g of clean silver metal in 10 ml of 1:1 nitric acid and dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Ag.

4.26 Stock Cadmium Solution

Dissolve 1.000 g of cadmium metal in concentrated hydrochloric acid. Dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Cd.

4.27 Stock Tin Solution

Dissolve 1.000 g of tin metal in 100 ml concentrated HCl and dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Sn.

4.28. Stock Barium Solution

Dissolve 1.779 g of barium chloride (dihydrate) in 1,000 ml of distilled water.
1 ml = 1,000 μ g Ba.

4.29. Stock Gold Solution

Dissolve 1.000 g of gold metal in a minimum of aqua regia. Take to dryness, dissolve the residue in 50 ml HCl and dilute to 1,000 ml with distilled water. Store in an amber bottle.
1 ml = 1,000 μ g Au.

4.30. Stock Thallium Solution

Dissolve 1.303 g of thallium nitrate in distilled water. Dilute to 1 liter with distilled water.
1 ml = 1,000 μ g Tl.

4.31. Stock Lead Solution

Dissolve 1.000 g of lead metal in concentrated nitric acid. Dilute to 1,000 ml with distilled water.
1 ml = 1,000 μ g Pb.

4.32. Lanthanum Solution (10%)

Dissolve 117 g of lanthanum oxide (high purity) in 200 ml concentrated hydrochloric acid. Dilute to 1,000 ml with distilled water.

4.33. 5% Potassium Solution

Dissolve 259 g potassium nitrate in distilled water and dilute to 2 liters.
1 ml \approx 50 mg K.

4.34. All stock standards are prepared in house from ANALAR or equivalent grade materials and verified by comparison to existing standards and/or standard reference materials.

4.35. Double distilled water. Lab distilled, deionized water is redistilled in a Corning all glass still.

Working standards are prepared by dilution of stocks, usually with 1% nitric acid plus 1000 mg/l K for flame atomic absorption. Typical concentrations of analytes in working standards range from 2.0 to 30 mg/l.

4.36. Mixed stock standards are normally used. The most common are listed below.

DO NOT EXCEED THESE CONCENTRATIONS AS
PRECIPITATION MAY OCCUR

Conc. mg/l

Matrix: 0.1% HNO₃ in double distilled water

Ni	100
Zn, Cu, Pb, Cd, Ag	40
Mn, Co, Cr (trivalent only)	40
Al, Fe	200
Li	20

For high volume filter digests, the following matrix
matched mixed standard is used.

Matrix: radiation buffer:

Ca 1500 mg/l
Na 1100 mg/l
Al 1000 mg/l
Mg 280 mg/l
K 80 mg/l
all in .3% HNO₃

The analytes are added to the matrix solution.

Zn, Pb, Mn, Ni, Cu, Fe	10
Cd, Cr, Be, V	1
Al	10

5. Procedure

This section catalogues the procedures routinely used to open out trace metals from all environmental matrices. The purpose of the digestion is to provide an aqueous, usually acidic solution containing the analytes of interest as simple aquated ions. The analytes must be stable in the digestate and the procedure should eliminate organics and other possible interferents.

Any wet digestion process is effectively a leaching procedure and as such may not necessarily solubilize all the analyte in any particular sample type. Volatilization losses may also occur. Cases where less than true "totals" are provided are detailed below.

5.1. Pretreatment of Liquid Samples

5.1.1. No Treatment

Acid precipitation leachates (5% HNO₃), field filtered throughfall, stemflow and lysimeter leachates (1% HNO₃), and other volume limited samples are analyzed as received by graphite furnace.

Precipitation samples are sealed in their collector bags in the field and submitted as is. The precipitation is analyzed as received

according to 5.1.2. The empty bag is leached with 1 L of 5% HNO_3 and the leachate is analyzed by graphite furnace.

Fe, Cu, Zn, Pb, Cd, Cr, Mn, Al, V, Ni are routinely determined by this procedure. Most other elements can be determined in special circumstances. Quality Control protocol is as in 5.1.2.

5.1.2. Preconcentration

This procedure is employed for all "clean" surface and drinking waters.

The digestion is suitable for all elements listed except tin which precipitates as an oxide in the presence of nitric acid.

CAUTION: All acid digestions must be carried under well ventilated conditions such as a fume hood.

5.1.2.1. The sample bottle is shaken and 100 ml poured into an acid rinsed digestion tube calibrated at 100 ml.

5.1.2.2. The acid washing procedure consists of a vigorous detergent wash followed by a distilled water rinse. The tubes are then filled with 5% HNO_3 and heated at 90° overnight, rinsed with distilled water followed by double distilled water and put into immediate use.

5.1.2.3. Samples are usually grouped in batches of 40. Each batch contains four reagent blanks, two control standards and two duplicates.

5.1.2.4. Batches are placed in a well ventilated oven at 95°C where they are allowed to evaporate to a small volume.

5.1.2.5. The samples are cooled and transferred to calibrated polystyrene centrifuge tubes. Potassium (0.1 ml) is added as 5% KNO_3 . The digestion tube is rinsed with small amounts of double distilled water and the rinsings are transferred to the centrifuge tube until a volume of 5.0 ml is reached.

5.1.2.6. The samples are mixed and the tubes sealed with parafilm to await analysis.

5.1.2.7. If significant residue is observed, the digestate is diluted to 10 ml. If undissolved material is still present, the sample is discarded and redone using the more vigorous aqua regia procedure.

NOTE: The precipitates are invariably, primarily calcium salts. Calcium must be reanalyzed on an undigested acidified aliquot.

5.1.3. Hydrochloric -Nitric Evaporation

5.1.3.1. Clean effluents.

5.1.3.1.1. Shake the sample and transfer a 50 ml aliquot to a test tube calibrated at 50 ml and 10 ml.

- 5.1.3.1.2. Evaporate the sample to dryness in an oven at 250°C.
- 5.1.3.1.3. Add 0.5 ml concentrated HNO₃ and 1.5 ml concentrated HCl to the tube and heat in an aluminum block to incipient dryness.
- 5.1.3.1.4. Dilute with distilled water to the 10 ml mark. Mix well and filter through a Whatman #40 filter into a disposable plastic tube.

5.1.3.2 Raw Sewage

- 5.1.3.2.1. Shake the sample and transfer a 25 ml aliquot to a test tube calibrated at 25 ml.
- 5.1.3.2.2. Evaporate the sample to dryness in an oven at 250°C.
- 5.1.3.2.3. Add 1.0 ml concentrated HNO₃ and 3 ml concentrated HCl to the tube and heat on an aluminum block to incipient dryness.
- 5.1.3.2.4. Dilute with distilled water to the 25 ml mark. Mix well and filter through a Whatman #40 filter paper into a disposable plastic tube.

5.1.3.3. Sludges

- 5.1.3.3.1. Transfer a 5 ml aliquot to a tube calibrated at 25 ml.
- 5.1.3.3.2. Follow steps 5.1.3.2.2. to 5.1.3.2.4.

5.1.3.4. Filter cakes

- 5.1.3.4.1. Weigh 30.0 g into a Waring blender. Add 270 ml distilled water. Blend until homogeneous.
- 5.1.3.4.2. Transfer a 5 ml aliquot to a tube calibrated at 25 ml.
- 5.1.3.4.3. Follow steps 5.1.3.2.2. to 5.1.3.2.4.

NOTE: Run format and protocol areas in 5.1.2. except only 2 reagent blanks are used.

5.1.4. Aqua regia digestion

This is the method of choice for heavy matrix samples not amenable to the preceding procedures. It is suitable for all elements except silver where sulphuric, nitric acid digestion is required.

- 5.1.4.1. A 10 ml aliquot, or less if the sample is very dirty, is poured into a 250 ml beaker.

- 5.1.4.2. Hydrochloric acid (conc. 3ml) and nitric acid (conc. 1 ml) are added and the beaker is covered with a watch glass. If necessary, distilled water is added to produce a total volume of ≈ 50 ml.
- 5.1.4.3. The sample is digested on a hot plate to a final volume of about 5 ml. If colored material still persists at this time it may be removed by the careful addition of 2 - 3 drops conc. HNO_3 .
NOTE: Very vigorous reaction occurs upon addition of HNO_3 .
- 5.1.4.4. The samples are removed from the hot plate and allowed to cool. The watch glass and cover are washed down with about 10 ml distilled water and the beakers are returned to the hot plate, brought to boiling and removed. The purpose of this procedure is to dissolve any slowly soluble salts.
- 5.1.4.5. The samples are allowed to cool and are transferred by rinsing with distilled water to a 50 ml digestion tube calibrated at 25 and 50 ml. The sample is made up to with distilled water. The tube is covered with parafilm, mixed by inversion and submitted for analysis.
- 5.1.4.6. Run format and protocol are as in 5.12 except only two reagents blanks are used.

5.1.5. Sulphuric-Nitric Acid digestion

This procedure is used for heavy matrix samples requiring silver analysis. It is also suitable for all routine elements except barium, calcium, gold, magnesium and tin. The run protocol and procedures are identical to 5.1.4. with the following exceptions:

- 5.1.5.1 Sulphuric acid (2 ml, conc.) and nitric acid (5 ml, conc.) are used.
- 5.1.5.2. The sample is digested until copious white fumes are evolved. If colored material still persists at this time it may be removed by the careful addition of 2 - 3 drops concentrated HNO_3 .

NOTE: Very vigorous reaction occurs upon addition of HNO_3 .

NOTE: The preceding procedures usually provide accurate "total" metal analysis of the sample. The treatments do not solubilize silicates, and some other minerals, however, and if substantial quantities of residue remain after digestion it is possible that biased low values may be obtained for aluminum, iron, titanium, chromium, calcium, magnesium and vanadium.

5.2. Sediments

5.2.1. Hydrochloric-Nitric Digestion

This procedure is used for the routine determination of all trace elements in sediments except silver which forms an insoluble chloride.

It is recognized that the digestion procedure does not bring all metals into solution. Varying proportions of the metals may be "locked" into some silicate minerals which are not dissolved (or "leached") by aqua regia. This dictates the need for close control of digestion conditions. It is felt, however, that the metals released into solution are of greater environmental significance than true "total" metals.

Rapid (day) and overnight procedures have been developed for the digestion of samples with hydrochloric/nitric acids. Unless results are required urgently the overnight procedure is preferred on the bases of reduced operator time and improved precision. Good organization of dried samples and clean glassware is essential for efficient operation of the "overnight" procedure.

5.2.2. 1-Day Digestion

5.2.2.1. Place about 15 g of well mixed wet sediment sample in a evaporating dish using a plastic spatula.

5.2.2.2. Dry sample in drying oven overnight at 100°C or air dry for several days.

5.2.2.3. Grind sample using mortar and pestle and sieve through 2 mm nylon sieve onto paper. Pour contents into 1 ounce vials. Discard paper and thoroughly clean sieve, mortar and pestle.

5.2.2.4. Weigh and 1.00 g portions of each sample and place in 125 ml Phillips beakers. Reference samples are also weighed in the same manner.

5.2.2.5. Place beaker on hot plate and add 10 ml concentrated HNO_3 and 20 ml to the sample. Add anti-bumping granules to each beaker. Two reagent blanks of each quantity of acid are prepared in the same way.

NOTE: Adding nitric acid first will minimize foaming. Samples should stand for about 30 minutes until foaming ceases.

5.2.2.6. Turn the hot plate to its minimum setting. Watch the beakers closely until the brown fumes are replaced by colorless vapour and the volume is about 2 ml. Remove each beaker from the hot plate as soon as digestion is complete and allow to cool. If foaming occurs, remove the beaker from hot plate and add 2 drops amyl alcohol to break the foam.

5.2.2.7. Add an additional 5 ml HCl to the beaker washing down the sides and return it to the hot plate. Remove when all brown fuming has ceased. Allow to cool. Caution must be taken to prevent "popping" of sample.

5.2.2.8. Fold filter papers and place in a pre-assembled battery of funnels. Wet down the filter paper with distilled water and place test tube under the funnel. Pour digestate into funnel and wash beaker with distilled water. Rinse the beaker again and allow precipitate to drain. Wash beaker once more into funnel, then wash down filter paper. Allow to drain. Remove test tube and funnel from rack and place in

test tube rack. Remove filter paper and wash down under side of filter paper and funnel into test tube.

- 5.2.2.9. Make solution to 50 ml volume with distilled water and cover with parafilm. Thoroughly mix the sample by inverting and shaking and submit for analysis.

5.2.3. Overnight Digestion

- 5.2.3.1. Weigh 0.500 g of finely powdered (< 10 mesh) sample into a 200 mm x 25 mm test tube, calibrated at 25 ml.

NOTE: FOR ALL STEPS AFTER THIS, WEAR SAFETY GLASSES AND PROTECTIVE GLOVES.

- 5.2.3.2. Add 1.5 ml of concentrated HNO_3 to the test tube, using an automatic dispenser. Allow to react for two hours at room temperature.

- 5.2.3.3. Add 4.5 ml of concentrated HCl , using an automatic dispenser. If excessive foaming occurs, add two drops of amyl alcohol. Allow to react for one hour at room temperature.

- 5.2.3.4. Transfer the test tubes to an aluminum block at ambient temperature.

- 5.2.3.5. Place the aluminum block on a hot plate, and slowly raise the temperature to 90°C - 100°C and leave at this temperature overnight or until 1 - 2 ml of acid are left.

NOTE: (1) If the content of any tube goes to dryness, add 0.5 ml of concentrated nitric acid and 1.5 ml of concentrated HCl and heat for one hour.

(2) If the temperature is raised too rapidly, some types of sample may splatter out of the tubes. If this occurs, the entire sample run must be repeated.

- 5.2.3.6. Allow the samples to cool to room temperature. Adjust the volume to 25 ml with distilled water. Mix well.

- 5.2.3.7. Filter about 15 ml of the solution through a dry Watman #40 filter into a clean test tube.

- 5.2.3.8. Analyse the solutions by atomic spectroscopy (ICAP or AAS).

5.3. Sewage and Sewage Sludge

5.3.1. Aqua Regia Digestion

The procedure is acceptable for most trace metals with the exceptions noted in 5.2.1 where a sulphuric-nitric procedure is required. Most sewages are routinely analyzed for Zn, Cu, Ni, Cd, Pb, Mo, Ca, Mg, Fe, Al, Co, Ti.

As has been discussed previously, the hot acid digestion may not provide true total results for all elements.

- 5.3.1.1. For wet sludges and effluents, shake bottle by hand. Mix thick and/or non-homogeneous sludges on a mechanical shaker or tumbler, or by blender and/or ultrasonic probe (Titanium Tip).

NOTE: Ensure good mixing action during mechanical shaking. Horizontal shaking can cause separation into layers.

Dewatered cakes can be weighed and resuspended in an appropriate measured volume of distilled water (usually 1:10) and blended for a few minutes with the Waring Blender. This dilution must be included in the calculation.

- 5.3.1.2. Transfer 5 ml by sludge pipette into digestion tube in tube rack and wash down sides of tube with distilled water. If the sample is too thick to pipette, weight out portion into tube. As it will not be practical to weigh out exactly 5 g, make the appropriate adjustment in calculation of dilution.
- 5.3.1.3. Place rack of tubes include controls in oven at 90°C and dry overnight.

Transfer tubes to aluminum block.

- 5.3.1.4. It is expedient to separate relatively clean samples from dirty samples in separate hot blocks.

Dispense 1 ml nitric acid into each tube followed by 3 ml of hydrochloric acid.

Digest on medium heat until brown fumes cease to evolve and smooth boiling and refluxing is evident (about 30 minutes for "cleaner" samples). If appreciable dark residue remains in the tube, add a few drops of nitric acid and continue refluxing until essentially clear. Cool and dilute to 25 ml with distilled water. Mix on the Vortex mixer, filter, and submit for analysis.

5.3.2. Sulphuric Nitric Digestion

Used for elements not amenable to the aqua regia digestion, e.g. silver and titanium.

The procedure is identical to the preceding aqua regia digestion with the following exceptions.

- 5.3.2.1. Add 1 ml sulphuric acid and 3 ml of nitric acid. Digest until copious white fumes. If digestion is incomplete, add more acid and continue. Cool, dilute and filter as for steps 5.2.2.8. and 5.2.2.9.

5.4. Vegetation

5.4.1. Aqua Regia Digestion

The procedure is employed for the majority of vegetation samples and is acceptable for all trace metals except silver which is rarely analyzed in vegetation. Studies have shown that for most normal vegetation all metals are extracted.

- 5.4.1.1. 1.00 g sample is weighed into a Vicor 30 ml crucible. It is placed in a muffle furnace at a temperature below 150°C. The temperature is raised to 250 °C, and held for 1 hr, then raised to 500°C and held for 3 hr.

- 5.4.1.2. 3 ml aqua regia (3:1 hydrochloric:nitric) is added and the sample is digested at moderate heat to a small volume.
- 5.4.1.3. The digestate is transferred to a culture tube marked at 10.0 ml, made up to volume and shaken. After settling, it is shaken again (this is necessary to avoid stratification within the tube).
- 5.4.1.4. Determinations are then made by AAS or ICP.
- 5.4.1.5. Run format and analysis protocol are identical to 5.1.2.

5.4.2. **Perchloric-Nitric-Hydrochloric Digestion**

This procedure is preferred for garden vegetables and forage samples which are quite resistant to digestion. It is not suitable for the determination of potassium because of the low solubility of potassium perchlorate.

NOTE: PERCHLORIC ACID IS POTENTIALLY EXPLOSIVE AND MUST BE USED WITH EXTREME CARE. IT MUST BE USED ONLY IN AN APPROVED WASH DOWN STAINLESS STEEL HOOD. EYE PROTECTION MUST BE WORN.

The procedure is identical to the preceding aqua regia method except the ashed residue is digested at moderate heat with a mixture of .5 ml hydrochloric acid, 1 ml nitric acid and 2 ml perchloric acid.

NOTE: HOT CONCENTRATED PERCHLORIC ACID IS EXPLOSIVE WHEN IN CONTACT WITH ORGANIC MATTER. IF ANY CHAR-RING OCCURS AS THE DIGESTION PROCEEDS NITRIC ACID SHOULD BE ADDED IMMEDIATELY AS A DILUENT AND THE SAMPLE REMOVED FROM THE HOT PLATE.

5.5. **Soils**

5.5.1. **Perchloric-Nitric Digestion**

The procedure is used for the determination of all trace metals except potassium in soils. It will not provide total values for elements bound in silicate matrices or certain refractory oxides. The data produced is considered to be more environmentally significant than true total values.

- 5.5.1.1. A 1.00 g aliquot of soil which has been previously air dried, ground and sieved is weighed into a 100 ml beaker.
- 5.5.1.2. 4 ml concentrated perchloric acid and 2 ml concentrated nitric acid are added and the sample is digested slowly at moderate heat to white fumes.

NOTE: PERCHLORIC ACID IS DANGEROUS. SEE NOTES ABOVE.

- 5.5.1.3. The digestate is allowed to cool and transferred by rinsing with distilled water into a 25 ml volumetric flask. The contents are well mixed two times and submitted for analysis.
- 5.5.1.4. Run format and quality control protocol are as described previously (5.1.2).

5.6. Biomaterials

This classification includes all animal tissue, e.g. fish, crayfish, clams, mink etc. About 90% of all determinations of samples in this classification are whole fish or fish tissue.

The procedure is suitable for the solubilization of all requested parameters; aluminum, calcium, cadmium, chromium, cobalt, copper, iron, lead, magnesium, manganese, nickel, zinc. These elements are totally extracted by the digestion process.

NOTE: Cr is volatilized if the sample is taken to a very small volume. (< 2 ml). Extreme care must be taken if this element is required.

- 5.6.1. An appropriate aliquot of tissue (usually 2.5 g) is weighed into a calibrated 50 ml digestion tube.

NOTE: Grinding samples prior to digestion increases precision but introduces contamination from the stainless steel blades, producing biased data for iron, nickel, chromium, manganese, and vanadium. Unground, "snip" samples are therefore used whenever possible.

- 5.6.2. Nitric acid (5 ml), perchloric acid (2 ml) and glass boiling beads are added to the samples and the mixture is allowed to sit and pre-digest at room temperature for several hours.

- 5.6.3. Samples, blanks and controls are then placed in a Technicon hot block and digested at 120°C for 2 hr followed by 170°C for 4 hr.

This treatment normally removes all nitric acid and produces a clear solution and heavy white fumes.

NOTE: PERCHLORIC ACID IS VERY DANGEROUS. SEE PRECAUTIONS 5.1.2.

- 5.6.4. If graphite furnace analysis is required for some parameters it is necessary to remove almost all the residual perchloric acid. To accomplish this, heating at 170°C is continued with careful attention until the samples are at incipient dryness.

- 5.6.5. The tubes are removed from the block, allowed to cool, brought to 25 ml with double distilled water, well mixed and submitted for analysis.

- 5.6.6. Quality control and run protocol are as previously described (5.1.2.).

5.7. Air Particulates

Air particulates are trapped on several types of filter media each of which require different digestion procedures.

Although the procedures are acceptable for many elements, those routinely requested and reported are zinc, copper, nickel, lead, cadmium, chromium, iron, vanadium, manganese. Currently 90% of all filters submitted are 8" x 10" glass fibre.

5.7.1. Hydrofluoric acid digestion: glass fibre filters

The procedure is used for the nine routine metals. Recent work on standard reference air particulates has shown the HF digestion to produce quantitative recoveries for all the above except chromium. Copper results will be biased high to a variable degree because of contamination from the motor exhaust of the Hi-vol apparatus.

- 5.7.1.1. Cut 3/4" x 10" strip from the glass fiber filter.
- 5.7.1.2. Ash in a muffle furnace, start cold and take up to 500°C for 2 hr.
- 5.7.1.3. After ashing, place in a Teflon dish on a hot plate set at 190°C. Add 15 ml of 1:1:1/HNO₃:HF:H₂O solution. Allow the solution to evaporate slowly.

Use stirring rod (Teflon) to break up filter.

Take to complete dryness.

NOTE: The evaporation temperature is critical. Overheating will produce premature volatilization of HF, causing the formation of a dense white precipitate.

NOTE: Residual HF can cause difficulties in the subsequent analytical step. Ensure all samples are taken to complete dryness in each step.

- 5.7.1.4. Add 5 ml of concentrated HNO₃, take to complete dryness.
- 5.7.1.5. Add 1 ml of concentrated HNO₃, take to complete dryness.
- 5.7.1.6. Repeat step 5.7.1.5.
- 5.7.1.7. Add 20 ml of 5% HNO₃ and evaporate down to 10 ml. Remove from hot plate, let cool and transfer completely into a 150 x 18 test tube marked at 15 ml. Make up close to this mark. Wash dish several times and scrape with Teflon scraper to remove all the salts.
- 5.7.1.8. Put the tubes in the aluminum block and place on a hot plate at 70°C overnight. This allows the salts to go into solution.
- 5.7.1.9. Remove next day. Solution must be crystal clear. Made to volume (15 ml), cover with parafilm and shake vigorously.
- 5.7.1.10. The sample is submitted for analysis.
- 5.7.1.11. Quality control and sample protocol is as previously described.

5.7.2. Nitric acid extraction - Glass fiber filters

This procedure provides a rapid quantitative extraction of lead from glass fiber filters and is used when lead is the only parameter requested for analysis.

- 5.7.2.1. With a #11 cork borer, punch out one circle from a folded filter. Sample size is 0.80 sq. inch.
- 5.7.2.2. Place the sample in 150 x 18 test tube in an aluminum block.
- 5.7.2.3. Add 1 ml of 50% v/v HCl.
- 5.7.2.4. Take to dryness on a hot plate at 120°C.
- 5.7.2.5. Add 1 ml of HNO₃ (conc.) and heat for 1 hour.
- 5.7.2.6. Allow to cool and make up to 15 ml.
- 5.7.2.7. Cover with parafilm and shake vigorously until sample forms a mash.

5.7.2.8. Centrifuge mash until clear then submit for atomic absorption analysis.

5.7.2.9. Analytical protocol, run formats and quality control are as previously described (5.1.2).

5.7.3. Hydrochloric Nitric Digestion - Whatman 41 Hi-Vol Filters

Whatman 41 filters are cellulose acetate. Thus the entire filter is removed by ashing and the relatively simple digestion procedure described below may be employed.

Of the nine routine elements, chromium results will likely be less than total.

5.7.3.1. Cut a 3/4" by 10" strip from the filter.

5.7.3.2. Place in a 100 ml glass beaker and cover with a watch glass.

5.7.3.3. Put in a furnace below 500°C until complete ashing occurs. Usually 3 hours.

5.7.3.4. When cool, lift the watch glass gently and with an eyedropper, wet the sample down completely with distilled water.

5.7.3.5. Take to the fume hood and add 5 ml of HCl (conc.). Place on the hot plate and at medium heat take to dryness, then remove from the hot plate.

5.7.3.6. Add 5 ml of HNO₃ (conc.) and again heat to dryness.

5.7.3.7. Add 2 ml HNO₃, heat, swirl and remove from the hot plate.

5.7.3.8. Transfer quantitatively into a 15 ml graduated test tube. Rinse the beaker several times with distilled water and bring up to the mark with washing.

5.7.3.9. Submit for analysis.

5.7.3.10. Quality control and analytical protocol are as previously described (5.1.2).

5.8. Andersen Head Filters

5.8.1. Hydrochloric Nitric Acid Digestion

5.8.1.1. Wash glassware (50 ml or 100 ml graduated beakers) in hot 10% HNO₃, rinse well with distilled water.

5.8.1.2. Cut 1/4 of the filter for analysis (pie shape).

5.8.1.3. Carry a reagent blank through each step.

5.8.1.4. Place filters in beakers, wet them with about 2 ml of water. Use disposable pipette for holding down and stirring.

5.8.1.5. Set the hot plate at 200°F, add 10 ml of HCl to the samples and take them to dryness.

5.8.1.6. Add 8 ml of HNO₃, and again take to dryness.

5.8.1.7. Add 20 ml of 1N HNO₃, and heat for a few minutes. Agitate with pipette, make to 50 ml in the beaker.

5.8.1.8. Decant about 10 ml of the extract through a #40 Whatman filter into test tubes.

5.8.1.9. Analyze by AAS for heavy metals per request.

5.8.1.10. Run format, analytical protocol and quality control are as previously described (5.1.2).

NOTE: Very vigorous reaction occurs upon addition of HNO_3 .

5.9. Delbag Filters

5.9.1. Hydrochloric Nitric Acid Digestion

5.9.1.1. Cut a 3/4" x 10" sample strip using a template and scalpel.

5.9.1.2. Place in a 100 ml beaker.

5.9.1.3. Ashing

5.9.1.3.1. Place the beakers in numerical order into the furnace.

5.9.1.3.2. Heat at 300°C for 1.5 hrs.

5.9.1.3.3. Raise temperature to 500°C for 1 hr.

5.9.1.3.4. Samples look greyish in color when properly ashed.

5.9.1.3.5. Further ashing may be necessary if the sample does not appear completely ashed.

5.9.1.4. Acid Extraction

Transfer the beakers to the hot plate and add 0.5 ml of HCl (conc.). Heat until just dry.

5.9.1.4.1. Add 0.5 ml HNO_3 (conc.). Heat until the initial reaction stops, then add 5 ml water.

5.9.1.4.2. Heat until the solution is clear.

5.9.1.4.3. Transfer quantitatively into the 18 x 150 mm test tubes (use policeman and wash 3 times).

5.9.1.4.4. Make to 15 ml mark when cool.

5.9.1.4.5. Shake well, then let settle. Samples are now ready for atomic absorption analysis.

NOTE: Very vigorous reaction occurs upon addition of HNO_3 .

5.10. Dustfall

The samples arrive in sealed polyethylen bags which are heated in an ultrasonic bath then separated into "soluble" and "insoluble" portions by suction filtration. (For more details see the Determination of Dustfall.)

5.10.1. Insoluble Fraction

5.10.1.1. Ash the sample at 500°C in a 50 ml beaker for 3.5 hours.

5.10.1.2. Add 5 ml conc. HCl and take to dryness at 95°C.

5.10.1.3. Add 5 ml conc. HNO_3 and take to dryness at 95°C.

5.10.1.4. Cool, add 1 ml HNO_3 and 2 ml of water. Heat to simmer for 3 minutes.

5.10.1.5. Cool and transfer to a calibrated 15 ml test tube. Wash out the beaker with small quantities of water which are added to the tube. Make up to the mark with distilled water.

5.10.1.6. Mix well.

5.10.1.7. Submit for atomic absorption analysis.

NOTE: Very vigorous reaction occurs on addition of HNO_3 .

5.10.2. **Soluble Fraction** - dried in a 250 ml beaker

5.10.2.1. Add 5 ml 1:1 (HNO_3 : H_2O) to the 150 ml beaker containing the sample.

5.10.2.2. Swirl the beaker so as to contact all residue on the sides of the beaker.

5.10.2.3. Heat gently on a hot plate, evaporating to about 1 ml.

5.10.2.4. Add 10 ml water. Heat at incipient boiling for about 10 minutes.

5.10.2.5. Cool, transfer to a calibrated 15 ml test tube.

5.10.2.6. Rinse and add the washings to the tube and bring to 15 ml with distilled water.

5.10.2.7. Mix well.

5.10.2.8. Submit for atomic absorption analysis.

5.11. Analysis of Prepared Solutions by Flame Atomic Absorption

Because of the large number of different instruments in use it is impractical to give a detailed description of the set up of each.

There are general principles which are followed however:

5.11.1. Pre-start Check

5.11.1.1. Cylinders securely fastened

5.11.1.2. Exhaust ventilation operating.

5.11.1.3. Burner head clean and securely fastened.

5.11.1.4. Water trap and/or loop filled.

5.11.1.5. Heater on if nitrous oxide is to be used.

5.11.1.6. Filters clean and dry.

Set up for individual parameters is generally in accord with manufacturer's recommendations. Pertinent information is contained in Table 1.1.

5.11.2. Parameters checked daily and optimized if necessary.

5.11.2.1. Lamp current alignment.

5.11.2.2. Instrument gain.

5.11.2.3. Burner alignment height.

5.11.2.4. Flame stoichiometry.

5.11.3. The absorbance of a check standard is analyzed and compared to previous data. If the value is within $\pm 10\%$ of the mean of previously amassed data the analyst proceeds.

5.11.4. The instrument is calibrated using mixed standards which encompass the normal range of samples (see 4.36).

- 5.11.5. Reagent blanks, control standards and samples are analyzed. When run manually, calibration is checked after every 20 samples and the blank checked with distilled water between samples. The PE 5000 automated procedure employs a distilled water blank after every 7 samples and 3 undigested check standards in the 50 position carousel.
- 5.11.6. Samples with concentrations greater than the highest standard are diluted to fall within the working range and reanalyzed.
- 5.11.7. The analytical values are either transcribed manually from the digital readout or captured by microcomputer.

6. Calculation and Reporting

The data from all instruments emerges in the form of concentration. Conversion to final reported results is handled somewhat differently in the manual and automated systems.

6.1. Manual:

The machines are constantly adjusted to a distilled water zero, thus the number obtained from the digital readout is the actual analyte concentration in solution. This is converted to the final result by the following:

$$R = (C_s - RBl) \cdot DF$$

Where:

R = final result

C_s = concentration in solution

RBl = average reagent blank

DF = dilution factor from the preparation step.

6.2. Automated System:

Prior to analysis, sample identifiers and dilution factors are entered into the PET microcomputer interfaced to the PE 5000 automated AAS.

The run format includes a distilled water blank after every 7 samples and a check standard at the beginning, middle and end of the run (as well as calibration standards etc.). The auto zero and reslope functions on the PE 5000 are not used. After the run is finished the PET corrects for baseline drift by linear interpolation between distilled water blanks. Slope corrections can also be made by the PET at the operator's discretion. The operator then enters the reagent blank value to be subtracted for each element after inspection of the raw data.

The PET then provides separate Quality Control and dilution corrected, blank corrected final reports using the method of calculation previously described.

Two significant figures are reported except in the decade containing the detection limit where one significant figure is used.

e.g. For detection limit = .003
0.5372 is reported as 0.54
0.0094 is reported as 0.009
0.0013 is reported as <.003

7. Precision and Accuracy

See Table 1.1.

8. References:

1. A Handbook of Decomposition Methods in Analytical Chemistry: Rudolf Bock, Iain L. Mars. T. & A. Constable Ltd., Edinburgh 1979.
2. Decomposition Techniques in Inorganic Analysis: J. Dolizall, P. Povondra, Z. Salchek. American Elsevier Publishing Co. Inc., New York 1968.
3. Atomic Absorption Spectroscopy: Gary D. Christian, Frediro J. Feldrman. Wiley-Interscience, New York 1970.
4. Standard Methods for the Examination of Water and Wastewater, 14th Edition. American Public Health Association, Washington, D.C. 1976.
5. Analytical Methods for Atomic Absorption Spectrophotometry. Perkin Elmer Corp., Norwalk, Conneticate 1976.

THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

Method 2

Flameless Atomic Absorption Spectrophotometry

SUMMARY

Matrix.	Precipitation samples only.
Substances Determined	Al, V, Cr, Mn, Fe, Ni, Cu, Cd, Pb (routinely).
Interpretation of results.	Results reported as the element in mg/l or $\mu\text{g}/\text{filter}$.
Principle of method.	A small aliquot of mildly acidic aqueous solution is injected into the cavity of a graphite tube furnace where it is dried, ashed and atomized by resistive heating. Atoms in the elemental state absorb energy of very specific wavelengths which are different for each element. An atom of aluminum, for example, will absorb energy provided by a hollow cathode lamp which emits the elemental spectrum of aluminum. The energy absorbed is directly proportional to the concentration of atoms in the original system.
Time required for analysis.	A single analysis requires about 1 minute. Because of standards, controls and set up time, about 150 samples can be analyzed per day. Preparation time varies greatly depending on sample type.
Range of application.	See Table 2.1.
Standard deviation.	See Table 2.1.
Accuracy.	See Table 2.1.
Minimum volume of sample.	2 ml.
Interferences and shortcomings.	As previously described, AAS is relatively interference free. The tube atomization step is subject to chemical thermodynamic and kinetic interferences. These can be minimized by skillful choice of temperature program. More important, only very "clean" samples are analyzed by this procedure where these effects are insignificant.

**Preservation and
sample container.**

1% HNO_3 in acid washed plastic bottles. Special procedures are used for precipitation. (See Method 1, 5.1.1.)

**Safety
considerations.**

Eye and hand protection must be worn when using concentrated acids. Concentrated acids should only be dispensed in a fume hood. The atomic absorption unit should have a proper exhaust canopy to expel the heat and fumes. Ensure that the gas cylinders are properly secured to the bench top. Ensure that the proper instructions for the operation of the atomic absorption unit are followed.

TABLE 2.1
FLAMELESS ATOMIC ABSORPTION
Concentration values are in mg/l

PE 603 and HGA 500

Matrix	Element	Detection Limit	Top of Range	Precision Between Run	Accuracy	Notes
1% HNO ₃ Optimum	Al	.005	.2	.029 at .219	100%	Platform Furnace
	Be	.002	.1			
	Cr	.0005	.02	.0001 at .0006	102%	
	Cd	.0001	.02			
	Co	.001	.03			
	Cu	.001	.2	.001 at .019	106%	
	Fe	.0006	.2	.003 at .043	110%	
	Mn	.0004	.05	.002 at .026	108%	
	Mo	.002	.2			
	Ni	.001	.2	.003 at .047	98%	
	Pb	.001	.2	.004 at .056	100%	
	Ti	.002	.1			
	V	.002	.1	.012 at .223	95%	1 Peak Area Peak Area

Precision Between Run: Standard deviation at level given of EPA #2SRM

Accuracy: Ratio of $\frac{\text{Mean EPA soln} \times 100}{\text{Design Value of EPA}}$

NOTE: 1 Background Correction

THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

Method 2

Flameless Atomic Absorption Spectrophotometry

1. Introduction

The basic difference between flame and flameless AAS is that the atomization process is conducted in a resistively heated graphite tube in an inert atmosphere rather than a flame. The tube furnace assembly is placed in the burner chamber such that the hollow cathode source passes down the tube cavity. An aliquot of sample (usually 20 μ l) is injected into the furnace via an autosampler through a small hole in the body of the tube.

The tube furnace is connected to a computer controlled power supply such that it can be electrically resistively heated under well controlled conditions. The sample is dried, ($\approx 100^\circ$ for 20 sec.), ashed ($\approx 700^\circ$ for 30 sec.) and atomized ($\approx 2500^\circ$ for 8 sec.). The tube is contained in a cell flushed with argon to prevent cell burning and increase atomization efficiency. The high temperature volatilizes metal salts and oxides. These compounds are subsequently reduced in the vapour phase to atoms which are measured by conventional AAS as described in Method 1. There are several advantages and disadvantages of this technique relative to flame AAS which dictate the sample types for which it is amenable.

An inherent sensitivity 10 to 50 times greater than flame atomic absorption and very small sample requirements (usually 20 μ l) are the major advantages. It therefore finds application for samples requiring low detection limits with limited sample volume. These conditions often exist for precipitation samples.

2. Interferences and Shortcomings

Because of the complexity of the volatilization, atomization process, the relatively long atomic residence times and non steady state conditions the technique is more susceptible to chemical interferences than flame atomic absorption. Different compounds have different lattice energies which will produce variable volatilization rates. Atomic species may recombine to form molecules in the gas phase producing decreased signals and background problems. Acid concentrations greater than 5% greatly decrease tube life and degrade precision and sensitivity. The interferences can often be decreased by judicious choice of ashing temperature, ramp rates, atomization temperature etc. Automated standard additions is possible for all but the very worst cases. In our laboratory, the technique is usually restricted to clean sample types such as precipitation, which have been previously shown to have no significant interferences and can be analyzed as received.

3. Apparatus

- 3.1. Perkin Elmer HGA 500 Graphite Furnace
- 3.2. AS 40 Autosampler
- 3.3. PE 603 Atomic Absorption Spectrophotometer.
- 3.4. See Method 1, 3.2 - 3.18

4. Reagents

See Method 1, 4.7 - 4.36 as appropriate.

5. Procedure

- 5.1. Samples are normally analyzed as received. See Method 1, 5.1.
- 5.2. Instrument set up and parameters are as per manufacturer's instructions. For individual elements see Table 2, Method 2.

NOTE: Because of the extremely low levels of analyte, cleanliness is essential. The instrument area is cleaned daily. Double distilled water is used in all operations.

- 5.3. A standard of known concentration is analyzed and the absorbance compared against the mean of previously acquired data.

NOTE: The graphite tubes deteriorate with use (lifetime 200 - 300 firings). Wearing is indicated by lower absorbance and/or decreased precision. A 20% reduction in sensitivity or within run precision of 7 - 10% is grounds for tube rejection.

- 5.4. After installation, new tubes are decontaminated and conditioned by several blank firings at maximum temperature.
- 5.5. The interior of the furnace is cleaned with ammonium hydroxide:acetone: water, 1:1:5 at the time of tube changing. The quartz windows are cleaned and furnace alignment is checked at the same time.

NOTE: Contact rings are changed if precision is bad with a new tube. The surface may be pitted. Rings are usually good for 30,000 firings.

- 5.6. Sample cups are manufacturer's issue. They are cleaned prior to use by soaking overnight in 10% KNO_3 followed by a double distilled water rinse and drying. Cups are discarded after use.
- 5.7. The run format for the first carousel of the day consists of air, standard blank, 8 matrix matched standards usually 1% or 5% nitric acid, 2 EPA standard reference standards, a composite control, air and 21 samples. Subsequent carousels (35 positions) are air, standard blank and 2 standards, 30 samples.
- 5.8. Readings in absorbance are transcribed by a printer.
- 5.9. The drying stage is visually observed about every 30 samples to ensure smooth and complete drying.

NOTE: Unfamiliar samples and complex matrices such as biological materials are handled much differently. Effectively, procedures must be tailored to each individual sample. The technique is very powerful, but it is not universally applicable.

6. Calculation and Reporting

Calculations are done via a program which calculates concentrations from a standard curve constructed from a least squares fit to a second order polynomial constrained to pass through an operator defined blank.

Dilution factors, significant figures etc., are handled as previously described. (Method 1, Section 6.)

7. Precision and Accuracy

See Table 2.1.

8. References:

1. A Handbook of Decomposition Methods in Analytical Chemistry: Rudolf Bock, Iain L. Mars. T. & A. Constable Ltd., Edinburgh 1979.
2. Decomposition Techniques in Inorganic Analysis: J. Dolizall, P. Povondra, Z. Salchek. American Elsevier Publishing Co. Inc., New York 1968.
3. Atomic Absorption Spectroscopy: Gary D. Christian, Frediro J. Feldrman. Wiley-Interscience, New York 1970.
4. Standard Methods for the Examination of Water and Wastewater, 14th Edition. American Public Health Association, Washington, D.C. 1976.
5. Analytical Methods for Atomic Absorption Spectrophotometry. Perkin Elmer Corp., Norwalk, Connecticut 1976.
6. The H6A 500 Graphite Furnace. Perkin Elmer Corporation, Norwalk, Connecticut 1980.

TABLE 2.2

METHODOLOGY - FAAS PE 603 - HGA 500

Element	λ (n.m.)	S.W.	Char. Temp. (°C)	Atom.	Detection Limit (ppm)	Sensitivity (ppm)	Working Range (ppm)	Comments
Zn	213.9	0.7	-	-	0.002	0.019	0.002 - 0.200 0.20 - 2.00	Flame - too sensitive by furnace
Cu	324.7	0.7	900	2700	0.001	0.0005	0.001 - 0.200	
Ni	232.0	0.2	1000	2700	0.001	0.001	0.001 - 0.200	
Pb	217.0	0.7	400	2300	0.001	0.001	0.001 - 0.200	Background correction
Cd	228.8	0.7	250	2100	0.0001	0.0001	0.0001 - 0.020	
Mn	279.5	0.2	1100	2700	0.001	0.0004	0.001 - 0.050	
V	318.4	0.2	1700	2800	0.005	0.0007	0.005 - 0.100	Peak area integration
Al	309.3	0.7	1400	2700	0.005	0.007	0.005 - 0.200	
Fe	248.3	0.2	1100	2700	0.001	0.0006	0.001 - 0.200	
Be	234.9	0.2	1200	2800	0.002	0.001	0.002 - 0.100	Peak area
Ti	365.4	0.2	1800	2800	0.002	0.0007	0.002 - 0.100	Peak Area Purge gas flow = 165 cc/min.
Cr	337.9	0.7	1100	2700	0.0005	0.0005	0.0005 - 0.020	
Co	240.7	0.2	1000	2700	0.001	0.001	0.001 - 0.030	
Mo	313.3	0.2	1800	2800	0.002	0.002	0.002 - 0.200	
As	193.7	0.7	250	2700	0.005	0.004	0.005 - 0.200	
Se	196.0	0.7	350	2700	0.005	0.004	0.005 - 0.200	
Bi	223.1	0.2	900	2300	0.005	0.004	0.005 - 0.200	

Purge gas flow = 50 cc/min.
(Argon)

Dry Temp = 100° Char. Time = 10 sec.
Dry Time = 20 sec. Atom. Time = 10 sec.

THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

Method 3

Inductively Coupled Plasma Atomic Emission Spectrometry

SUMMARY

Matrix	Surface water, seaage, sludges, soilsand, sediments, vegetation, air particulates and biomaterials.
Substances determined.	Al, As, Ba, Be, B, Ca, Cd, Co, Cu, Cr, Fe, Mg, Mn, Mo, Ni, P, Pb, S, Sn, Ti, V, Zn. (Others by special request.)
Interpretation of results.	Results reported as the element in mg/l or other appropriate unit.
Principle of method.	An aliquot of the sample is prepared so as to render it free from organics and suitable for aspiration into a plasma discharge. (See Method 1.5.1) The plasma serves as an atomization device as well as a source of excitation energy. The atoms emit their characteristic radiation and the energy of this radiation indicates which element is present; the intensity indicates the amount of analyte present. A computer controlled spectrometer is used to analyze the plasma discharge and concentrations are printed directly.
Time required for analysis.	A single analysis requires about 1½ minute. Because of standards, blanks, controls and setup time, about 150 samples (i.e., 3000 tests) can be done per day. Preparation time varies greatly with sample type.
Range of application.	See Table 3.1.
Standard deviation.	See Table 3.1.
Accuracy.	See Table 3.1.
Minimum volume of sample.	A single analysis consumes only 2.5 ml of solution. Much larger volumes (or weights) may be required, however. See Table 1.2.

**Detection
criteria.**

See Table 3.1.

**Interferences and
shortcomings.**

ICP methods are relatively free of matrix problems but do suffer from spectral interferences when very low concentrations of one element are being measured in the presence of a high concentration of another. These effects can be overcome in most cases by computer controlled inter-element corrections, but the analyst must be completely informed about the nature of the sample and the analytical requirements.

**Preservation and
sample container.**

See Table 1.2.

**Safety
considerations.**

Extreme care should be taken in the handling of acids, especially perchloric. A well ventilated fume hood is required. Wear eye protection at all times when digesting samples or handling acids. The ICP spectrometer must be well grounded and proper ventilation should be used. Cylinders should be secured. Since potentially lethal voltages are being employed and since the equipment is extremely complex, the operator must be properly trained and experienced in all safety related aspects.

TABLE 3.1

JY 48 ICP ATOMIC EMISSION

(all values are in mg/l)

Matrix	Element	Detection Limit	Top of Range	Precision Between Run	Accuracy*	Notes
Preconcentrated	Al	.02	350	1.44 ± .05	+0.01 at .4	
Aqueous Acid	As	.05	400		-.003 at .06	
Digests	B	.005	180	1.56 ± .05		
5% HNO ₃	Ba	.002	40	1.25 ± .03		
Optimum	Be	.002	2	1.75 ± .06	-.01 at .2	
	Ca	.02	300	2.02 ± .19		
	Cd	.002	30	1.80 ± .04	-.002 at .03	
Surface water	Co	.005	70	1.79 ± .05	-.003 at .10	
and Drinking	Cr	.005	50	1.75 ± .03	-.003 at .06	
Waters	Cu	.005	50	1.63 ± .02	+0.006 at .05	
	Fe	.008	150	1.74 ± .04	-.003 at .08	
	Mg	.08	400	2.02 ± .03		
	Mn	.002	17	1.81 ± .04	+0.001 at .05	
	Mo	.005	40	1.65 ± .03		
	Ni	.01	60	1.92 ± .06	+0.003 at .10	
	P	.1	1300	1.10 ± .08		
	Pb	.05	300	1.73 ± .05	-.003 at .11	
	S	.1	120			
	Se	.1	400		+0.001 at .02	
	Sn	.1	400			
	Sr	.005	30	1.27 ± .01		
	Ti	.005	25	4.58 ± .06		
	V	.005	50	1.31 ± .04	-.02 at .5	
	Zn	.002	40	1.72 ± .05	-.003 at .03	
Aqueous acid	Al	1	2000			
Digests of	As	.5	400			
Industrial	B	.5	180			
Wastes and	Ba	.1	40			
Contaminated	Be	.05	20			
Waters	Ca	1	3000			
	Cd	.01	30			
	Co	.05	70			
	Cr	.05	120			
	Cu	.05	150			
	Fe	5	1200			
	Mg	1	1000			No SRM's Available
	Mn	.1	40			
	Mo	.05	40			
	Ni	.05	60			
	P	1	1300			
	Pb	.06	300			
	S	.1	180			
	Sr	.5	30			
	Ti	.1	75			
	V	.1	50			
	Zn	.05	100			

* EPA #2 Water 10x preconcentration.

TABLE 3.1 (con't.)
JARRELL ASH ATOMCOMP 975 ICP ATOMIC EMISSION*
(all values are in mg/l)

Matrix	Element	Detection Limit	Top of Range	Precision Between Run	Accuracy*	Notes
Inhouse Reference Water						
Aqueous Acid Digests of Whatman Filters	Al	.05	500	4.62 ± .9	No SRM's Available	
	Be	.02	20	1.23 ± .076		
	Cd	.006	50	.95 ± .17		
	Cr	.02	80	1.61 ± .094		
	Cu	.01	80	4.59 ± .383		
	Fe	.03	500	4.65 ± .236		
	Mn	.006	80	.601 ± .033		
	Ni	.05	80	.501 ± .03		
	Pb	.1	200	1.51 ± .085		
	V	.02	20	1.12 ± .085		
Zn	.05	80	1.55 ± .21			
Inhouse Reference						
Aqueous Acid	Al	.08	500	77 ± 3.8	No SRM's Available	
Sewage Sludges	As	.08	200	4.3 ± .23		
	Ca	.06	2000	208 ± 11.5		
	Cd	.01	50	7.16 ± .38		
	Cr	.03	80	7.7 ± .34		
	Cu	.02	80	4.79 ± .24		
	Fe	.05	500	258 ± 10.5		
	Mg	.01	1000	40 ± 1.8		
	Mn	.01	80	6.31 ± .25		
	Mo	.05	50	12.5 ± .64		
	Ni	.07	80	1.7 ± .09		
	Pb	.13	200	1.98 ± .028		
	Zn	.08	80	16.1 ± .81		

TABLE 3.1 (con't.)
JARRELL ASH ATOMCOMP 975 ICP ATOMIC EMISSION*
(all values are in mg/l)

Matrix	Element	Detection Limit	Top of Range	Precision Between Run	Accuracy*	Notes
Inhouse Reference						
Aqueous Acid Glass Fiber Filters	As (B)	.05	200	4.3 ± 2.3		No SRM's Available
	Ba	.02	40			
	Be	.02	20			
	Ca	.04	2000	208 ± 11		
	Cd	.0006	50	7.2 ± 4		
	Co	.02	50			
	Cr	.02	80	7.7 ± .3		
	Cu	.01	80	4.8 ± .2		
	Fe	.03	500	258 ± 10		
	Mg	.01	1000	40 ± 2		
	Mn	.0006	80	6.3 ± .2		
	Mo	.03	50	12.6 ± .5		
	Ni	.05	80	1.7 ± .1		
	Pb	.1	200	1.98 ± .3		
	Sr	.01	60			
	Ti	.1	200			
	V	.02	20			
	Zn	.05	80	208 ± 11		
	Inhouse Reference					
Preconcentrated	Al	.05	500	.34 ± .04	+0.05 at .852	
Aqueous Acid	As	.05	200	.012 ± .006	.02 at .18	
Digests of	Be	.02	20		+0.02 at .26	
Precipitation	Ca	.04	2000			
5% HNO ₃	Cd	.0006	50	.008 ± .0008	+0.0008 at .059	
Optimum	Co	.02	50	.003 ± .002	+0.02 at .34	
	Cr	.02	80		.041 at .304	
	Cu	.01	80	.074 ± .004	+0.013 at .374	
	Fe	.03	500	.36 ± .02	+0.109 at .796	
	Mg	.01	1000			
	Mn	.0006	80	.074 ± .002	+0.032 at .478	
	Mo	.03	50	.002 ± .003		
	Ni	.05	80	.048 ± .008	+0.019 at .165	
	Pb	.1	200	.010 ± .001	.008 at .383	
	V	.02	20	.007 ± .002	+0.019 at .867	
	Zn	.05	80	.46 ± .02	+0.011 at .478;	

* EPA #2 Water 20x Preconc.

* In order to ensure accuracy it is necessary to calibrate the instrument with standards which approximate the analyte and background element concentrations. The table contains information on routinely used matrices.

THE DETERMINATION OF TRACE METALS By Atomic Spectroscopy

Method 3

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP)

1. Introduction:

Atomic emission spectroscopy is a widely used analytical technique for the analysis of trace metals in environmental samples. As in atomic absorption, an aqueous sample, or aqueous acid digestate is aspirated into an inductively coupled argon plasma. The plasma is extremely hot (9000°K), and thus totally disrupts any molecules, and atomizes and excites all components in the samples. When the excited atoms and ions return to lower energy states, they emit light of very specific wavelengths. The wavelengths for each element are characteristic and well known. The coherent emitted light is separated by a diffraction grating. Phototubes are located at specific locations relative to the grating to intercept the wavelength for each analyte of interest. The photomultiplier counts are monitored under computer control, the data is massaged as described below, and results are printed out in concentration. In contrast to atomic absorption, where elements are determined individually, here over 20 elements are determined on a single aspiration. Sensitivities and detection limits are comparable or superior to atomic absorption.

2. Interferences and Shortcomings:

Because of the extremely high temperature of the argon plasma, chemical interferences as described in atomic absorption literally do not occur. Sample introduction problems can happen, usually caused by saturated salt solutions (e.g. KClO_4) forming deposits on the nebulizer tip, producing partial clogging and severe sensitivity and precision changes. This problem is handled by dilution or, preferably, by avoiding the use of perchloric and sulphuric acid in digestion procedures.

Acid concentration should be consistent and no greater than 10% V/V. Higher concentrations adversely affect precision. One to five per cent is optimum.

Very high concentrations of one element can produce spectral interferences on another. For example, 2138 Å is the primary Zn line. However, there is a weaker Zn line at 2148.9 Å which interferes with the P line at 2149.0 Å. As well, some scattered light from the primary Zn line reaches the P phototube. Both effects are predictable and repeatable, and thus can be estimated by a computer controlled interelement correction factor (IEC). This factor for Zn on P on the JY 48 is .053. Thus, if the machine finds 10 mg/L Zn, it will automatically subtract $10 \times .053 = .53$ mg/L from the measured P result.

Scattered light, emission from the torch and several other factors can produce background emission leading to erroneously high results. Our instruments have the capacity to measure the background intensity at a selected distance from the analytical line and correct by subtraction.

NOTE: One must be extremely careful in the choice for background measurements. Choice of a location where another spectral line is found would produce an anomalously high correction.

3. Apparatus:

- 3.1 Inductively coupled plasma emission spectrometers Jarrell Ash .75 m Atomcomp model 975, Jobin-Yvon 1 m JY48P
Each of the above equipped with autosamplers DEC computers capable of direct concentration printouts and interfaced to PET microcomputer systems for further data massage, storage and transfer.
- 3.2 See Method 1 from 3.2 - 3.18.

4. Reagents:

See Method 1 from 4.1 - 4.36.
Mixed stock standards are used as described in Table 3.2.

5. Procedure:

5.1. Sample preparation: See Method 1 from 5.1 - 5.7

Run format and quality control format are generally as described in the Sample Preparation Section (Method 1.5.2). Added machine controls are described below.

5.2. Analysis of Prepared Solutions by ICP Spectrometry

The following is a guide to the operation of the JY48P. The parameters are to be used only as a guide. The optimum conditions must be worked out for each instrument and sample type. Similar procedures are followed for the Jarrell Ash 975.

The following detailed procedure is applicable only to the JY48 and is included to provide an indication of the complexity of operation of the instrument.

Procedure for Routine Analysis

- 5.2.1. Turn on RF generator.
- 5.2.2. Turn on Ar cylinder valves.
- 5.2.3. Turn on all three Ar lines, (plasma, neb, and aux); allow nebulizer to aspirate d.w. for 1-2 minutes then turn off "neb" Ar.
- 5.2.4. Adjust N₂ purge to 3 l/min.
- 5.2.5. Turn on cooling water.
- 5.2.6. Place system disk in L.H. drive of RX02.
- 5.2.7. Place data disk in R.H. drive of RX02.
- 5.2.8. Check that terminal is ON.
- 5.2.9. "BOOT" the system disk by raising the single-position toggle switch on the lower right side of the computer.
- 5.2.10. The computer will print:
28 START enter DY
- 5.2.11. Enter the date and time in response to the queries.
- 5.2.12. •R JY48P.SAV (wait 20-30 sec. until program is loaded)

- 5.2.13. Enter current position, then peak position.
- 5.2.14. The computer will print out a string of options: suspend operations at terminal and proceed to step 5.2.15.
- 5.2.15. Light the plasma and start the nebulizer (turn OFF aux).
- 5.2.16. Nebulize a solution of 1000 ppm Y and ensure that red cone is adjusted to reference point . . . return sample tube to d.w.
- 5.2.17. Proceed back to terminal and enter PR
- 5.2.18. Enter table filename, e.g. DAVID
- 5.2.19. The computer will print out a string of options: enter CH
- 5.2.20. Enter 17 (17 is the PCN for Mn)
- 5.2.21. Nebulize a solution of ≈ 10 ppm Mn.
- 5.2.22. Ensure that green scanning motor drive switch is in OFF position.
- 5.2.23. Manually move profile adjust knob so that you can take a reading (at equal intensity values) on each side of the Mn line.
- 5.2.24. The average of the two readings from 5.2.23 is the profile maximum position.
- 5.2.25. Turn on the green scanning motor drive switch.
- 5.2.26. *Use the "MOVE" option to move the profile meter to the position of maximum intensity.
* (You may have to initialize the λ drive by alternately pushing the red then the white then the green then the white switches.)
- 5.2.27. Use the "INI" option to inform the computer of the position of maximum intensity.
- 5.2.28. END (the profile routine).
- 5.2.29. The computer will respond with a string of options; enter AN
- 5.2.30. The computer will respond with ANA CHG END
- 5.2.31. Type CHG and enter "21" as an output device.
- 5.2.32. Type ANA
- 5.2.33. The computer will respond with a string of options; enter ASBSBGACACNCO
- 5.2.34. The computer will ask you how many samples you are running; enter the number of standards to run
- 5.2.35. Enter these identifiers:
BLK
CAL001
CAL002
CAL003
etc.
- 5.2.36. Run these solutions and check intensity values as they are printed out vs. tabulated values.
- 5.2.37. When these solutions have been run, the computer will print out a table of parameters . . . Check vs. previous values.
- 5.2.38. The computer will print out ANA CHG END Enter CHG . Change output device 1; then enter ANA
- 5.2.39. The computer will respond with a string of options; enter ASBSBGACNDFCO
- 5.2.40. Enter total no. of solutions that you will be running (including reagent blanks and standards).
- 5.2.41. Enter default dilution factor.
- 5.2.42. Enter your analytical run. A typical run would look something like this:
DDWBI
BLK 1, BLK 2 (reagent blank)
CHECK 1
CHECK 2

SAMPLE #1
SAMPLE #2
...
SAMPLE #10
DDWBI
CHECK #1
CHECK #2
etc.

- 5.2.43. One or more of the "samples" are "check" standard(s) for which the concentration is accurately known; the results will check the quality of the preparation stage.
- 5.2.44. The "CHECK" samples are in-house references which are used exclusively to check instrument setup and drift.
- 5.2.45. The samples are then analyzed using the sensitivity ranges, IEC's and background correction angles previously determined to be optimum for the matrix to be analyzed.
- 5.2.46. Results are displayed on the screen as the run proceeds so the operator can monitor the system, detect and correct problems such as baseline drift, sensitivity change, etc.
- 5.2.47. Samples with concentrations beyond the range of the instrument are diluted and reanalyzed.
- 5.2.48. SHUT-DOWN:
 - i) Push BLUE rf OFF button
 - ii) Push plasma gas OFF (allow nebulizer to run in d.w. for awhile)
 - iii) Terminate program operation by entering END until computer prints "STOP . . . program terminated by operator".
 - iv) Remove disks and place in protective sleeves, place in plastic box.
 - v) Turn off λ scan drive; turn off water; turn off nebulizer; turn off Ar gas valves; turn off generator.

TABLE 3.2
MIXED STOCK STANDARDS*

Elements Present		Conc. mg/l	
Matrix: 1% HNO ₃ in double distilled water			
Surface Waters	#1	Ba, Mo, Ni, Sr	10
		Al, Fe	50
	#2	Cd, Cu, Co, Zn, Pb, Mn	10
		Ca, Mg	50
	#3	As, B, Cr	10
		P	50
	#4	V, Ti	10
		S	50
	#5	Be	10
	Matrix: 4% HNO ₃ in double distilled water, 2000 mg/l K		
Sewage Digests	#1	Ca	200
		Cu, Mn, Pb, Zn, Ni	10
		Cd	2
	#2	Al	100
		Fe	200
		Ca	10
		Mg	60
	#3	Cr, As	10
		Mo	2
	Matrix: 5% HNO ₃ in double distilled water		
Precipitation	#1	Ca, Mg	10
		Cd, Co, Cu, Mn, Pb, Zn	1
	#2	Al, Be, Fe, Mo, Ni	1
	#3	Cr	1
		As	2
	#4	V	1

MIXED STOCK STANDARDS* (Cont'd)

Elements Present		Conc. mg/l
Surface Waters	Matrix: 5% HNO₃ in double distilled water	
	#1 Mo, Ni, Ba, Sr	10
	Al, Fe	50
	#2 Cd, Co, Cu, Mn, Pb, Zn	10
	Ca, Mg	50
	#3 As, B, Cr	10
Glass Fibre Filter Digests	#4 Ti, V	10
	#5 Be	10
	Matrix: radiation buffer:	
	Ca 1500 mg/l	
	Na 1100 mg/l	
	Al 1000 mg/l	
	Mg 280 mg/l	
	K 80 mg/l	
	in .3% HNO ₃	
	#1 Zn, Pb, Mn, Ni, Cu, Fe	10
	Cd, Cr, Be, V	1
	Al	10
Matrix: 1% HNO₃ in double distilled water		
Surface Waters	#1 Ca, Cd, Cu, Co, Mg, Mn, Pb, Zn	10
	#2 Al, Ba, Be, Fe, Mo, Ni, Sr, Ti	10
	#3 As, B, Cr	10
	#4 Bi, V, Hg	10

* The concentrations used are chosen so as to approximate the relative expected concentrations of analytes.

These concentrations must not be exceeded as precipitation may occur.

6. Calculation and Reporting:

All calculations are done by the PET microcomputers according to the following protocol. Prior to analysis, sample identifiers and dilution factors have been entered into the system. After the run, all distilled water blanks significantly different from zero and all check standards are displayed and the operator applies linear interpolation, blank and slope drift corrections where appropriate.

All significant reagent blanks are then displayed and the operator, where necessary, enters the reagent blank value to be subtracted.

All Q.C. data is then stored on disc, as well as sample results. The final concentrations are then printed and/or transmitted directly to the laboratory central data handling computer.

7. Precision and Accuracy

See Table 3.1.

8. References:

1. A Handbook of Decomposition Methods in Analytical Chemistry: Rudolf Bock, Iain L. Mars. T. & A. Constable Ltd., Edinburgh 1979.
2. Decomposition Techniques in Inorganic Analysis: J. Dolizall, P. Povondra, Z. Salcek. American Elsevier Publishing Co. Inc., New York 1968.
3. Atomic Absorption Spectroscopy: Gary D. Christian, Frediro J. Feldman. Wiley-Interscience, New York 1970.
4. Standard Methods for the Examination of Water and Wastewates, 14th Edition. American Public Health Association, Washington, D.C. 1976.
5. Analytical Methods for Atomic Absorption Spectrophotometry. Perkin Elmer Corp., Norwalk, Connecticut 1976.
6. Tables of Spectral Lines: A.N. Zaidel et. al. IFI/Plenum Data Corporation, New York 1970.
7. An Atlas of Spectral Interferences in ICP Spectroscopy: M.L. Parsons, Alan Foster, Don Anderson. Plenum Press, New York 1980.

THE DETERMINATION OF TRACE METALS
By Atomic Spectroscopy

Method 4

D.C. Arc Atomic Emission Spectroscopy

4 - 1: Qualitative Method

Matrix.	The method may be used for virtually any sample type amenable to ashing.
Substance determined.	Total Cd, Ba, Be, As, B, Si, Zn, Sb, Ge, Tl, Mn, Mg, Pb, Sn, Cr, Fe, Bi, Al, V, Ca, Ti, Cu, Ni, Si, Co, Mo, Te, Ce, Ag.
Interpretation of Results	Results are reported as the element in mg/kg or percent.
Principle of method.	An aliquot of dried and ashed sample is mixed with graphite and radiation buffer and a subsample is packed in an electrode. An electric discharge is established between the sample and a counter-electrode which vaporizes, atomizes and excites the sample. The atoms return to lower energy states emitting characteristic energy which is dispersed by a grating and recorded as lines on a photographic plate.
Time required for analysis.	Depending on the ramification used, the time is highly variable. Normally about 10 samples can be weighed, mixed and "burned" in one day. Dilutions and plate interpretation can be very time-consuming, depending on the degree of precision and accuracy required of the result.
Range of application.	As required, usually .1 to 100 mg/kg for most elements (See Table 4.1).
Standard deviation.	Results reported as ranges. Typically a factor of 2 to 3, e.g. Fe 10 -30 mg/kg.
Accuracy.	Within the limits of precision of the experiment on SRM's, NBS Orchard Leaves, Tomato Leaves, Air Particulate. (see Table 4.1.)
Detection criteria.	Variable with element, technique, and the nature of the sample; from .1 to 1 mg/kg for most cases. (See Table 4.1.)
Interferences and shortcomings.	The procedure is insensitive for some elements (e.g. Zn) and has a narrow dynamic range, often requiring time-consuming dilutions. Because of the plate technique, one can use several lines to confirm the presence of an element and circumvent interferences.

**Minimum volume
of sample.**

A minimum of 0.1 g of ashed material is required for analysis. This may correspond to 1 liter of a clean water or .1 g of incinerator residue.

**Preservation and
sample container.**

See Table 1.2

**Safety
considerations.**

Extreme care is required for acid digestion procedures as previously described. The electrode arc should not be viewed directly. Care should be taken because of the high voltages used.

THE DETERMINATION OF TRACE METALS By Atomic Spectroscopy

Method 4

D. C. Arc Atomic Emission Spectroscopy

4 - 1: Qualitative Method

1. Introduction

The basic differences between ICPAES previously described and the DC Arc procedures are the source and detectors.

Here the source is a D.C. arc between two graphite electrodes, one of which contains the sample. The electric arc provides the energy for the vaporization, atomization and excitation processes. Note also that DC arc utilizes solid samples. A modification of this procedure using a rotating graphite electrode rotrode and an AC spark works directly with homogeneous liquids and slurries, such as waste oils.

The emitted light from the arc is dispersed by a grating onto a photographic plate. The plate containing the total spectrum of the sample is developed. Such spectra each contain several thousand lines. In contrast to the ICP direct reader, where a single phototube is placed to "catch" a single line for each analyte of interest, here we have a complete picture. One can locate several lines to confirm the presence of an element or know immediately if strong background emission is present in a particular region. Note that the emission spectrum will be different if a different source (e.g. rotrode) is used. As well, the emission spectra for all elements are well known, so it is possible to scan for rarely determined analytes such as Te or W. The plate also provides a permanent record which can be examined for further elements or rechecked at any time.

Because the procedure directly atomizes solids, it is not subject to the vagaries of acid digestion procedures and thus can provide true total metals data. This is most important for elements such as Si and Ti which routine digestion procedures will not bring into solution.

Both analytical procedures and preparative techniques are tailored to each individual sample type and problem. It would be impossible to list all the nuances within the scope of this manual. The two most commonly used procedures are dealt with in detail.

NOTE: Almost any sample type can be analyzed for any element. Prior discussion with the analyst is essential.

Qualitative Method:

A qualitative analysis is carried out when the absolute concentrations of the constituents are of secondary concern and when the overall composition of the sample is of interest. The method is often employed when there is more interest in what is not present. Using the following method, routine analysis of 30 elements on a qualitative basis is possible.

Semi-Quantitative Method:

Semi-quantitative determination generally involves an estimation of the content of the analytes by visual comparison with standards and is liable to subjective errors. An accuracy of ± 10 -20% may be expected at levels above the detection limit. The semi-quantitative analysis of algae or vegetation for 13 elements is possible on a routine basis; Cr, Ge, Ag, Be, Ti, Mo, V, Ni, Mn, Pb, Mg, Cu, Ba. The concentrations of another 7 elements can be estimated on the same sample (Sn, Cd, Bi, Zr, In, Tl, Co). However, the accuracy of these estimates is "order of magnitude" only.

2. Interferences and Shortcomings

During the drying and ashing stages of the procedure, some analytes (e.g., As, Se, Hg) may be partially lost. The technique is relatively slow and labour intensive. This is compounded by the relatively small dynamic range afforded by photographic detection, thus requiring many dilutions, which further slows production.

Background emission and spectral overlap, as mentioned in Method 3, do occur, but are readily circumvented by subtracting a background component or using alternative lines. Because of these options, DC arc has excellent accuracy. The photographic plate, however, is much less precise than the phototube. At best it is $\pm 10\%$. The most practical use of the method is for unknowns and preliminary surveys, where information about the fundamental nature and composition of a material is required.

3. Apparatus

- 3.1. Jarell-Ash 3.4 meter Ebert type convertible spectrograph/spectrometer combination. A 570 lines per millimeter plane grating blazed at 3000 Å provides a linear reciprocal dispersion at the focal surface of approximately 5 Å per millimeter over the spectral range 2000 Å - 4500 Å
- 3.2. Microphotometer
- 3.3. Graphite electrodes
- 3.4. Ceramic Mortar and pestle
- 3.5. Wigglebug

CAUTION: A well-ventilated area is required when working with graphite powder.

Also see Method 1 from 3.2 - 3.17.

4. Reagents

- 4.1. Mixed powder standards "Spexmix"
- 4.2. SPEX - Potassium sulphate K_2SO_4
- 4.3. ROC/RIC - Palladium diaminodinitrite $(NH_3)_2 Pd(NO_2)_2$
- 4.4. Ultra Carbon - Graphite 200 Mesh "F" purity
- 4.5. 50% Buffer - Pure K_2SO_4 (potassium sulphate) mix in a ratio of 1 to 1 with pure graphite.
- 4.6. Saturated solution of commercial sugar in ethanol.

5. Procedure:

- 5.1 Liquid samples are evaporated to dryness, usually in the presence of acid. The residue is then treated as a solid sample.

- 5.2 If significant organic appearing materials are present, the samples should first be dried at 100 °C, then ignited for 60 minutes at 250 °C, then for 3 hours at 500 °C.
 - 5.3 Each sample is prepared in a 2x dilution and a 100x dilution.
 2x dilution: 0.2000 gm of sample + 0.2000 gm of 50% buffer. Grind 1 minute with ceramic mortar and pestle and shake 30 seconds on the wiggiebug.
 100x dilution: 0.0100 gm of the 2x dilution + 0.4900 gm of the 25% buffer. Grind 1 minute with ceramic mortar and pestle and shake 30 seconds on the wiggiebug.
 - 5.4 Grind with mortar and pestle all samples and standards
 - 5.5 0.04 gm of the above mixes are packed into 10 mg Harvey type electrodes by inverting the electrode into the mix and against the glass ball in the vial. Add 2 drops of ethanol sugar mixture and pin. Dry at 160 °C overnight at 15" mercury in vacuum oven.
 - 5.6 Qualitative Standards
 Spex in graphite standards are diluted with pure K₂SO₄ to contain 25% K₂SO₄ in the final mix. (eg. 0.75 gm of spex/graphite standard + 0.25 gm of pure K₂SO₄). Standards are prepared by dilution of the "Spexmix" standards with appropriate amounts of the 50% buffer. Standards will contain 750, 225, 75, 22.5, 7.5 mg/kg.
- EACH STANDARD WILL BE GROUND FOR 1 MINUTE WITH CERAMIC MORTAR AND PESTLE.
- Two blanks are needed: one for 2x dilution and a second for the 100x dilution. Each will contain approximately 25% K₂SO₄ in the burn mix.
- 5.7 In order to minimize variability and facilitate plate reading, many control samples are used. The run protocol is:
 - 1% Fe (a 1:100 dilution of pure Fe powder)
 - 7.5 - 750 mg/kg mixed standards
 - 1% Fe
 - 100x diluted sample #1
 - 2x diluted sample #1
 - 75 mg/kg mixed standard
 - 1% Fe
 - 100x diluted sample #2
 - 2x diluted sample #2
 - etc.

Analysis

- 5.8 The samples are placed in the holder and "burned" by producing a DC arc between the electrodes.
- 5.9 The instrument is operated in accordance with the manufacturer's instructions.
- 5.10 The following parameters are routinely used.

Excitation:

15 Amp DC arc for 90 seconds

Argon/Oxygen ratio is 150/50

6 mm pointed counter electrode

6 mm analytical gap; adjusted back to 6 mm at 45 secs into burn.

Air chamber (environmental control chamber)

Exposure:

26 Microns width, 2 mm height

1.1 absorbance filter

50/30 rotating step sector

SA-1 plates developed 5 minutes in D19 at 68°C.

- 5.11 The developed plate which contains up to 30 "burns" is read by micro-photometer.

Intensities are estimated by visual comparison to the standards.

NOTE: Extreme care is required to ensure that the proper line is being read. Proper alignment is checked by reference to the 1% Fe spectra.

TABLE 4.1
QUALITATIVE DC ARC EMISSION SPECTROSCOPY
Analytical Data

<u>Element</u>	<u>Wavelength, Å°</u>	<u>Detection Limited mg/kg</u>	<u>Top of Range mg/kg</u>	<u>Precision</u>	<u>Accuracy (certified in brackets)</u>	<u>Notes</u>
Cd	2288	3	1000		1-10 (1)	Ref. Sediment #1
Ba	2335.27	3	1000			
Be	2348.61	0.5	30			
As	12349.84	30	1000			
B	2496.78	1	300			
	2497.73	1	300			
Si	2506.9	3	1000			
	2881.58	1	-			
Zr	3391.98	0.5	150			
Sb	2311.47	7.5	150			
Ge	2651.18	3	100			
	2651.58	7.5	-			
Tl	2580.14	3	1000			
	2767.87	3	-			
Mn	2576.12	0.5	1000			
	4033.07	0.5	-			
	4034.49	2.0	-			
Mg	2776.69	7.5	1000			
	2802.70	0.2	-			
Pb	2833.07	3.0	1000			
Sn	2839.99	2.0	300			
	2863.33	3.0	-			

Ref. Sediment #1

10-50 (25)

cont'd...

TABLE 4.1 (Cont'd)

QUALITATIVE DC ARC EMISSION SPECTROSCOPY
Analytical Data

Element	Wavelength, Å	Detection Limited mg/kg	Top of Range mg/kg	Precision	Accuracy (certified in brackets)	Notes
Cr	2843.25 4354.35	2.0 0.5	300 -			
Fe	3047.61 2395.63	3 7.5	- 1000			
Bi	3067.72	1	300			
Al	2652.49 2567.99	50 1	1000 -			
V	3102.30 3110.71	1 2	300 -		50-100 (60)	Ref. Sediment #1
Ca	3158.87 3179.33	3 2	1000 -			
Ti	3239.04	0.5	300			
Cu	3247.54 3273.96	0.1 0.1	60 -		50-100 (85)	Ref. Sediment #1
Ni	3050.82 3414.77	1.0 0.5	300 -		10-50 (30)	Ref. Sediment #1
Sr	3464.46	3	500			
Co	3474.02 2363.79	3 75	150 -		10-50 (25)	Ref. Sediment #1
Mo	3208.83	5	300			
Te	2385.76	75	1000			
Tl	3775.72	3	300			
Ce	4286.60	10	1000			
Ag	3280.68	.1	100			

6. Calculation and Reporting

A concentration range is determined by visual estimation of line darkening of the sample as compared with standards. A range, utilizing the standard concentrations, is recorded (e.g. Pb 0.2 - 0.6 mg/kg) in the burn mix. All results are reported as mg/kg or mg/L in the sample as received.

A sample calculation is given below:

2 g dried sample produces 0.4 g ash. 0.2 g of the ash are mixed with 0.2 g of 50% buffer. The sample concentration in the burn mix is estimated by visual comparison to the standards.

Pb: $0.4 \times 2 \times .4/2 = .16$ mg/kg dry weight in the sample.

2 = buffer dilution factor

.4/2 = loss on ignition factor

7. Precision and Accuracy

Results are usually reported as a range of a factor of three. Accuracy is within the limits of precision on several SRM's.

NBS Air Particulates

NBS Orchard Leaves

NBS Tomato Leaves

NBS Bovine Liver

See Table 4.1.

8. References.

- 8.1 Spectrochemical Analysis, 2nd Edition; L.H. Ahrens, S.R. Taylor.
Addison-Wesley Publishing Company Incorporated. Reading, Massachusetts
1961.

TRACE METALS

DC Arc Atomic Emission Spectroscopy

Method 4

4 - 2: Semi-Quantitative Method

Summary

Matrix.	Vegetation and filamentous algae.
Substance determined.	Cr, Ge, Ag, Be, Ti, Mo, V, Mn, Ni, Pb, Mg, Cu, Ba.
Interpretation of results	Results are reported as the element in mg/kg or percent.
Principle of method.	An aliquot of dried and ashed sample is mixed with graphite and radiation buffer and a subsample is packed in an electrode. An electric discharge is established between the sample and a counter-electrode which vaporizes, atomizes and excites the sample. The atoms return to lower energy states emitting characteristic energy which is dispersed by a grating and recorded as lines on a photographic plate.
Time required for analysis.	10 samples can be analyzed per day.
Range of application.	From .5 to 100 mg/kg. The range can be extended by dilution.
Standard deviation.	Varies somewhat with element. Usually 10% to 20% at 10 times the individual detection limit.
Accuracy.	Within the limits of precision of the experiment on NBS/Orchard Leaves and Tomato Leaves. See Table 4.2.
Detection criteria.	Variable with element: from .5 to 30 mg/kg. See Table 4.2.
Interferences and shortcomings.	The procedure is insensitive for some elements (e.g. Zn) and has a narrow dynamic range, often requiring time-consuming dilutions. Because of the plate technique, one can use several lines to confirm the presence of an element and circumvent interferences.
Minimum volume of sample.	5 g optimum; 0.5 g minimum.
Preservation and sample container.	See Table 1.2.
Safety considerations.	The electrode arc should not be viewed directly. The equipment is potentially dangerous. Appropriate training is required. Good ventilation is required when working with carbon.

Table 4.2

Semi-Quantitative DC Arc Data

Element	Wavelength	Detection Limit mg/kg	Top of Range mg/kg	Precision Within Run	Accuracy	Notes
Ag	3280.68	0.5	10	53± 16	+10 at 53	spike
Ba	2335.27	3	250.	564± 65	+16 at 500	spike
Bi	3067.72	5	75	50± 5	+6 at 50	spike
Be	3131.07	1	10.	43± 4	+1 at 13	NBS Fly Ash
Cd	2288.02	5	250	82± 8	+34 at 80	spike
Co	3453.51	1	50	NA	NA	
Cr	2843.25	3	150	42± 6	+13 at 130	NBS Fly Ash
Cu	3273.96	.5	22.5	136± 16	+1 at 12	Tomato leaves
Ge	2651.18	5	75	46± 6	+1 at 46	spike
In	3258.56	5	75	84± 8	+5 at 50	spike
Mg	2779.83	3	75	NA	+2 at .5	NBS cement
Mn	2933.06	5	100	430± 40	-60 at 490	NBS Fly Ash
Mo	3170.35	2	75	53± 7	+9 at 53	spike
Ni	3050.82	3	100	39± 4	+0 at 98	NBS Fly Ash
Pb	2833.07	3	150	48± 4	-5 at 70	NBS Fly Ash
Sn	2839.99	5	75	69± 10	+25 at 69	spike
Ti	3240.71	3	50	1700± 130	-300 at 2000	NBS cement
Tl	2767.87	5	50	NA	NA	
V	3102	5	75	99± 9	-14 at 214	NBS Fly Ash
Zr	3391.98	2.5	50	NA	NA	
B	2497.73	2	50	75± 10	+21 at 75	spike
Ga	2943.64	1	22.5	25± 2	+1 at 61	spike
Ca	3179.33	5	200	49± 2%	+2 at 47	NBS cement
Al	2652.49	30	750	2.7± 2%	+1 at 2.6	NBS cement
Fe	3050.82	5	500		-190 at 700	Tomato leaves

TRACE METALS DETERMINATION
DC Arc Atomic Emission Spectroscopy
Method 4

4 - 2: Semi-Quantitative Method

1. Introduction

See Method 4.1. - Introduction.

This procedure has in principle the same wide applicability as the Qualitative method. To this point, it has been applied to a limited number of elements in vegetation and filamentous algae. This method is described below. These limitations are not analytical so much as logistical, and in future, the method will certainly receive more diverse use.

2. Interferences and Shortcomings

See Method 4.1 - Interferences.

The lines and elements were selected because they were found to be interference free for the matrices in question.

3. Apparatus

See Method 4.1. - Apparatus.

4. Reagents

- 4.1 Mixed powder standards "Spexmix".
- 4.2 ROC/RIC Ultra Carbon Graphite 200 mesh "F" purity
- 4.3 Palladiumdiaminodinitrite $(\text{NH}_3)_2\text{Pd}(\text{NO}_2)_2$
- 4.4 SPEX - Potassium sulphate K_2SO_4 .
- 4.5 Ultra Carbon - Graphite 200 Mesh "F" purity.
- 4.6 50% Buffer - Pure K_2SO_4 mixed in a 1 to 1 ratio with pure graphite.

5. Procedure

Sample Preparation:

- 5.1 The algae or vegetation is separated from all extraneous material (sticks, sand, etc.) and dried at 80°C .
- 5.2 1.0 gm is weighed into a porcelain evaporating dish and placed in a muffle furnace. The following heating steps are used:
 - 15 min at 150°C
 - 60 min at 250°C
 - 3 hours at 500°C .

- 5.3 The loss on ignition is calculated.
- 5.4 The ash is diluted back to the original dried weight with graphite. (N.B. If an aliquot is taken for dilution, it must be ground in a ceramic mortar and pestle.)
- 5.5 The diluted material is further diluted by mixing 1:1 with quantitative buffer (25% K_2SO_4 in graphite with 0.010% $(NH_4)_2Pd(NO_2)_2$ so that the concentration in this burn mix is exactly one-half the concentration in the dried vegetation sample.
- 5.6 **THIS BURN MIX MUST BE GROUND IN A CERAMIC MORTAR AND PESTLE.**
- 5.7 0.020 gm of this mix are weighed into a 330902 graphite electrode and tamped into the electrode with a 4 mm diameter graphite rod.

Standard Preparation:

- 5.8 "Spexmix" standards are diluted to the appropriate concentrations with graphite and mixed 1:1 with quantitative buffer.

NOTE: The standards are prepared in large batches which usually last about one year. 0.02 g of the standards are used for each burn.

Analysis

- 5.9 See Method 4.1. from (5.8 - 5.11).
 - 5.10 Excitation:
 - 15 amp DC Arc for 90 seconds
 - Argon/Oxygen ratio is 150/50 (**DO NOT USE CHAMBER**)
 - 6 mm pointed counter electrode
 - 6 mm analytical gap; adjusted back to 6 mm at 45 sec into the burn.
 - 5.11 Exposure:
 - 10 micron width, 2 mm height
 - 0.5 absorbance filter
 - 50/30 rotating step factor
 - S A-1 plates developed 3 min in D19 at 68 °C.
 - 5.12 The intensities of the appropriate lines for samples and standards are measured by microphotometer. Absorbance versus concentration curves are constructed and sample concentrations in the burn mix are recorded.
- NOTE:** The microphotometer is interfaced directly to a PET microcomputer which does all the above.

6. Calculation and Reporting

Line intensities are measured by microphotometer and transmitted to a PET which produces concentrations of sample in the burn mix.

Final concentrations are calculated as below:

Conc. in the burn mix \times DF = Conc. in dried sample

DF = Dilution factor of the quantitative buffer (usually 2)

7. Precision and Accuracy

Both are estimated from replicate analysis of NBS Tomato Leaves, and other SRM's.
See Table 4.2.

8. References.

- 8.1 Spectrochemical Analysis, 2nd Edition: L.H. Ahrens, S.R. Taylor.
Addison-Wesley Publishing Company Incorporated, Reading, Massachusetts
1961.

THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

Method 5

X-ray Fluorescence Spectrometry

5 - 1: Lead in Air Particulate

SUMMARY

Matrix.	The method is used routinely for Pb determination in air particulate samples.
Substance determined.	Total Pb.
Interpretation of results.	Results are reported as $\mu\text{g}/\text{m}^3$ in air.
Principle of method.	The sample is subjected to X-ray photons from a chromium target X-ray tube. The fluorescent X-rays produced by the sample are diffracted by the analyzing crystal and are measured using an appropriate detector.
Range of application.	200 - 40,000 μg Pb per 20 cm x 25 cm filter corresponding to 0.1 to 20 $\mu\text{g}/\text{m}^3$ Pb in air for a 2000 m^3 air volume.
Standard deviation.	2.5% relative standard deviation (RSD).
Accuracy.	$\pm 12\%$ as compared to AAS method.
Detection criteria.	200 μg Pb/filter or 0.1 $\mu\text{g}/\text{m}^3$ Pb in air.
Interferences and shortcomings.	Arsenic interference is eliminated by measuring the Pb L β line. Matrix effects are partly compensated for by the calibration technique used.
Minimum volume of sample.	A filter disc 35 mm in diameter.

**Preservation and
sample container.**

Filters are stored in envelopes. Preservatives are not required.

**Safety
considerations.**

Normal safety precautions of an X-ray laboratory. Radiation monitoring badges must be worn.

THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

Method 5

X-RAY FLUORESCENCE SPECTROMETRY

5 - 1: Lead in Air Particulate

1. Introduction

An aliquot of filter is inserted into the spectrometer sample holder. The sample is subjected to X-ray energy from a chromium X-ray tube. The secondary X-rays produced by fluorescence are dispersed by a lithium fluoride crystal and measured by a scintillation counter. The number of counts is obtained for 2 successive 20 second periods. The lead concentration of the sample is determined from a previously prepared calibration curve.

Although chemical methods are available for the determination of lead in air particulates, lengthy acid pretreatments are required to obtain lead in solution. The X-ray fluorescence (XRF) spectrometric method, however, allows the detection of lead directly on the glass fibre filter without any chemical treatment. The analytical time for 1 sample is about 1.5 minutes and the sample is not destroyed during the analysis and can be stored for future reference. The XRF method is therefore used for the determination of lead in air particulate matter.

2. Interferences and Shortcomings

The strongest analytical line for measuring lead by XRF is the Pb L α line. However, any arsenic present in the sample will interfere positively with the determination of lead, since the As K α and the Pb L α lines coincide. The weaker Pb L β line is therefore used for analysis. Other interferences may be caused by a non-uniform dust deposit, by the particle size distribution, or by the matrix effects of other elements present in the dust.

3. Apparatus

- 3.1. X-ray Fluorescence Spectrometer, Siemens SRS or equivalent with scintillation detector and associated pulse-counting electronics.
- 3.2. Cutting die, with a diameter of at least 35 mm to excise an aliquot of filter.
- 3.3. Pellet press.
- 3.4. Balance.

4. Reagents

- 4.1. Lead chloride (PbCl_2), reagent grade.
- 4.2. Cellulose powder, ash free analytical filter pulp, S & S No. 289 (or equivalent).
- 4.3. Wax, a suitable grade is available from Hoechst Canada Ltd., Toronto, as Hoechst "C" wax.
- 4.4. **Lead Chloride Solution (2 g/l Pb)**
Dissolve 2.685 g lead chloride in distilled water and dilute to 1 liter.

4.5. Standard Pellets

- 4.5.1. Place 2.0 g cellulose powder in each of a series of 100 ml beakers and add 0, 5, 10, 15, 20, 30 and 40 ml volumes lead chloride solution to each beaker respectively, to give the following lead concentrations: 0.0, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 % by weight.
- 4.5.2. To each beaker, add sufficient distilled water to completely soak the cellulose powder, mixing each slurry well with a stirring rod. Dry contents of beakers in a drying oven at 50°C.
- 4.5.3. Add 0.5 g wax to contents of beaker and mix thoroughly. Transfer the cellulose-wax mixture to the die of a pellet press and pelletize material at a pressure of 25 tons for 30 seconds.
- 4.5.4. Repeat this procedure for each remaining standard. Label each pellet according to its weight concentration with respect to cellulose. These pellets are used as stability checks for the X-ray spectrometer from day to day.

5. Procedure

5.1. Standardization

- 5.1.1. Standardize the XRF method by comparing the counts obtained on a filter aliquot against the concentration of lead on the same filter as determined by atomic absorption spectrophotometry (AAS).
- 5.1.2. Select about 80 - 100 Hi-Vol glass fibre filters, 20 cm x 25 cm which have been exposed for up to 24 hour periods to collect air particulate, and which contain various amounts of lead in the 0 - 15,000 $\mu\text{g}/\text{filter}$ range.
- 5.1.3. Determine the number of counts on an aliquot of each filter by XRF (according to 5.2).
- 5.1.4. Analyze a known smaller portion of this aliquot for its lead concentration by AAS and determine the lead content of the filter.
- 5.1.5. Plot the line of best fit between the number of X-ray counts obtained and the lead concentration in $\mu\text{g}/\text{filter}$. Use this as a calibration curve. Under the conditions used, this curve is linear for lead concentrations up to 40,000 $\mu\text{g}/\text{filter}$.

5.2. Determination of Lead

5.2.1. Operating Conditions of Instrument

X-ray tube: chromium anode
Power: 50 kV, 40 mA
Collimator: 0.15 mm
Crystal: lithium fluoride 200
Analytical line: Pb L_{β} ($28.21^{\circ} 2 \theta$ angle)
Counting time: 2 x 20 seconds, with sample rotation
Vacuum: 0.5 Torr
Detector: scintillation counter
Pulse height analyzer
 setting: baseline 1.8 v; width 4.0 v
Sample holder: 34 mm diameter opening

- 5.3. Peak the X-ray spectrometer at the Pb L_{β} line using the standard lead pellet containing 0.5% lead.

- 5.4. Determine the number of counts for 2 successive periods of 20 seconds each, for each of the standard lead pellets.

NOTE: These pellets are used to check the instrument stability from day to day. If counts differ by more than 10% from values obtained at time of calibration, redo the calibration procedure.

- 5.5. Cut a circular aliquot of the sample filter using the cutting die.

- 5.6. Insert filter aliquot into the spectrometer sample holder with the dust-containing side facing up so that the clean unexposed side faces the X-ray tube.

NOTE: These instructions apply to the Siemens SRS spectrometer in which the X-ray tube is located below the sample holder. If a spectrometer having the tube above the sample holder is used, the dust containing side of the filter disc should face the tube.

- 5.7. Secure filter in holder with a retaining ring.

NOTE: To minimize the number of counts due to scattering, the ring should not be in the path of the X-ray beam.

- 5.8. Insert sample holder with filter into the X-ray beam and obtain the number of counts of 2 successive 20 second periods.

- 5.9. Repeat steps 5.5 - 5.8 for each sample analyzed. Also analyze 2 blank filter aliquots for each batch of samples. The blank filters should be from the same manufacturing batch as the exposed sample filters.

6. Calculation and Reporting

- 6.1. Determine the average number of counts for the 2 consecutive 20 second periods of counting for the exposed and blank filters. Subtract the average number of counts for the blank from that obtained for the sample to give the average number of net counts/20 seconds.

- 6.2. Using the average net counts for each sample, read the lead concentration from the calibration curve prepared as previously described.

NOTE: Alternatively, it may be convenient to use the equation of the line of best fit to calculate lead concentration on the filter. Under the instrumental operating conditions and the specific filters used for calibration the equation is:

$$\mu\text{g Pb on filter} = \frac{\text{average net counts} - \text{intercept}}{\text{slope}}$$

- 6.3. Calculate $\mu\text{g}/\text{m}^3$ Pb in air as follows:

$$\mu\text{g}/\text{m}^3 \text{ Pb} = \frac{\mu\text{g Pb on filter}}{\text{volume air sampled (m}^3\text{)}}$$

- 6.4. Results are reported to 1 decimal place.

7. Precision and Accuracy

The precision of the method, as obtained from the analysis of duplicate aliquots from a series of filters, is 2.5% RSD. The accuracy of the XRF method is $\pm 12\%$, as compared to the AAS method. Comparison of results obtained by the XRF and the AAS methods gives a correlation coefficient of 0.97.

8. Bibliography

- 8.1. Magyar, B. and Vonmont, H. (1976). Kombinierte anwendung der atomabsorption unter der roentgenfluoreszenz bei der bestimmung von blei in atomosphaerischem stab. Zeitschrift fuer Analytische Chemie **280**: 11-120.
- 8.2. O'Connor, B.H., Kerrigan, G.C., Thomas, W. and Gasseng, R. (1975). Analysis of the heavy element content of atmospheric particulate fractions using X-ray fluorescence spectrometry. X-ray Spectrometry **4**: 190-195.
- 8.3. Pimenta, J.A., Chan, C.C.Y. and Rees, G.S. (1977). A Rapid Method for the Determination of Lead in Suspended Air Particulate Matter - Preliminary Report. Laboratory Services Branch, Ministry of the Environment, Rexdale, Ontario.
- 8.4. Pimenta, J.A., Mace, V., Rees, G.S. and Roberts, P.J. (1978). The Determination of Optimal Parameters for the Analysis of Lead by X-ray Fluorescence Spectrometry. Laboratory Services Branch, Ministry of the Environment, Rexdale, Ontario.

THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

Method 5

X-ray Fluorescence Spectrometry

5 - 2: Calcium on CoH Tapes

SUMMARY

Matrix.	The method is used routinely for Ca determination in air particulate samples.
Substance determined.	Total Ca.
Interpretation of results.	Results are reported as $\mu\text{g}/\text{m}^3$ in air.
Principle of method.	The sample is subjected to X-ray photons from a chromium target X-ray tube. The fluorescent X-rays produced by the sample are diffracted by the analyzing crystal and are measured using an appropriate detector.
Range of application.	0.07 - 150 μg Ca per CoH tape spot corresponding to 0.1 to 200 $\mu\text{g}/\text{m}^3$ Ca in air for a 0.7 m^3 air volume.
Standard deviation.	5% (RSD).
Accuracy.	$\pm 8\%$ as compared to ICP atomic emission method.
Detection criteria.	0.07 $\mu\text{g}/\text{spot}$ or 0.1 $\mu\text{g}/\text{m}^3$ Ca in air.
Interferences and shortcomings.	The Ca $\text{K}\alpha$ line is free of analytical interferences. Deep penetration of the Ca particulate within the Whatman CoH tape results in the attenuation of the Ca fluorescence.
Minimum volume of sample.	A filter disc 35 mm in diameter.

**Preservation and
sample container.**

Filters are stored in envelopes. Preservatives are not required.

**Safety
considerations.**

Normal safety precautions of an X-ray laboratory. Radiation monitoring badges must be worn.

THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

Method 5

X-ray Fluorescence Spectrometry

5 - 2: Calcium on CoH Tapes

1. Introduction

The CoH tape filter is trimmed such that the deposition will be centered within the spectrometer sample holder. The sample is then irradiated using a Cr target X-ray tube. The fluorescent X-rays produced by the sample are diffracted by a PET crystal and measured by a flow proportional counter. The number of counts is obtained for 2 successive 20 second periods. The Ca concentration of the sample is determined from a previously prepared calibration curve.

2. Interferences and Shortcomings.

The $\text{CaK}\alpha$ line is free of analytical interferences. If the Ca particulate has penetrated deep within the filter, then the cellulose matrix of the filter will attenuate the Ca fluorescent radiation. This will produce an analytical result which is less than the true amount present. Based on intercomparisons with the ICP atomic emission, this does not appear to be a significant problem.

3. Apparatus

- 3.1. X-ray Fluorescence Spectrometer, Siemens SRS or equivalent with scintillation detector and associated pulse-counting electronics.
- 3.2. Cutting die, with a diameter of at least 35 mm to excise an aliquot of filter.
- 3.3. Pellet press.
- 3.4. Analytical balance.
- 3.5. Membrane Filtration Unit.

4. Reagents

- 4.1. Reagent grade CaF_2 powder.
- 4.2. Reagent grade Isopropyl alcohol.
- 4.3. 47 mm diameter nuclepore filters.
- 4.4. USP grade Collodion.
- 4.5. Amyl acetate (mixed isomers)

5. Procedure

5.1. Calibration Standard Preparation

- 5.1.1. Finely grind a 1 g sample of CaF_2 .

- 5.1.2. 0.1 g of the finely ground CaF_2 is added to 100 ml of isopropyl alcohol (IPA) and sonicated in an ultrasonic bath for 10 minutes. Agitate the suspension and resonicate. Repeat the last step an additional two times. Let the suspension settle. Decant the liquid and save. Weigh the sediment and thus determine the approximate concentration of CaF_2 suspended in the IPA.
- 5.1.3. Dilute a 10 ml aliquot of the above suspension to 1000 ml using IPA.
- 5.1.4. Filter a 200 ml aliquot of the suspension produced in 5.1.3. onto a preweighed (5 place) nuclepore filter. Let dry and reweigh. The concentration of the suspended particulate within the stock solution is then calculated.
- 5.1.5. Prepare a stock solution of 5% collodion in amyl acetate. Saturate the surface of four nuclepore filters with collodion by atomizing the 5% stock solution.
- 5.1.6. Filter 10, 20, 50 and 100 ml aliquots of the CaF_2 stock suspension through the four separate conditioned filters.
- 5.1.7. Prepare a stock solution of 0.1% collodion in amyl acetate. Lightly atomize the 0.1% collodion solution over the 4 standard filters. This step is performed so as to wet the particles and bind them to the heavier collodion layer below.
- 5.1.8. Prepare two "Daily Standardization Pellets" by adding arbitrary amounts of CaF_2 to cellulose and pelletizing as described in the procedure for the determination of Pb by XRF.

5.2. Calibration Procedure

- 5.2.1. Operating Conditions of the Instrument:
X-ray tube target: Cr.
Power: 45 kV, 30 mA.
Collimator: 0.4 mm.
Crystal: P.E.T.
Analytical line: $\text{CaK}\alpha(45.186^\circ 2\theta)$
Counting time: 2 x 20 sec. with sample rotation.
Vacuum: 0.5 Torr
Detector: Flow proportional counter
Pulse height analyzer setting: Baseline 1.55V, width 4.0 V.
Sample holder: 34 mm diameter opening.
- 5.2.2. Peak the X-ray spectrometer at the $\text{CaK}\alpha$ line using one of the daily standardization pellets.
- 5.2.3. Determine the number of counts for 2 successive 20 second periods for each of the standard pellets.
NOTE: These pellets are used to check the instrument stability from day to day. If the counts differ by more than 10% from values obtained at the time of calibration, recalibrate the instrument.

- 5.2.4. Add the 4 calibration standards (deposit side down) and determine the number of counts for two successive 20 second periods for each of the standards.
- 5.2.5. Prepare a calibration curve by plotting the average of the two 20 seconds counts vs the total mass of Ca on the filter. Obtain the least squares equation for the best fit through these points. Under the instrumental operating conditions and the specific filter used for calibration, the equation obtained was:

$$\mu\text{g Ca per filter} = \frac{(\text{Count} - \text{Blank}) - \text{INTERCEPT}}{\text{Slope}}$$

5.3. Routine Analysis

- 5.3.1. Load the sample chamber of the X-ray spectrometer with the two "Daily Standardization Pellets" and 8 blank CoH tape filters. Obtain two 20 second counts for each of the samples using the condition of section 5.2.1.

- 5.3.2. Calculate the Daily Standardization Factor (f) which is defined as:

$$f = \sum C_{is} / \sum C_{is}^o$$

Where:

C_{is} = counts obtained for the standardization pellets from section 5.3.1.

C_{is}^o = counts obtained for the standardization pellets from 5.2.3.

- 5.3.3. If $0.9 \leq f \leq 1.1$ then proceed to step 5.3.4; otherwise restandardize the instrument.
- 5.3.4. Calculate B which is the average of the counts for the 8 blank filters.
- 5.3.5. Load the sample chamber with the 10 filters to be analyzed and determine the number of counts for each filter as in section 5.3.1.

6. Calculation and Reporting

- 6.1. Calculate the concentration of Ca in air from:

$$(\text{Ca}) = ((C-B)f - b) / .776 a$$

Where:

C = counts obtained for the sample

B = average blank value (section 5.3.4.)

f = Daily Standardization Factor (5.3.2.)

b = intercept of the calibration curve

a = slope of the calibration curve

0.776 = air flow for a 2 hr CoH tape.

Results are expressed as $\mu\text{g Ca per cubic meter } (\mu\text{g}/\text{m}^3)$

7. Precision and Accuracy

The precision of the method as obtained from the analysis of duplicate samples is $\pm 5\%$. The accuracy of the XRF method is $\pm 8\%$ as compared to the ICP atomic emission spectrometer determination of Ca.

8. Bibliography

- 8.1. Magyar, B. and Vonmont, H. (1976). Kombinierte anwendung der atomabsorption unter der roentgenfluoreszenz bei der bestimmung von blei in atomosphaerischem stab. Zeitschrift fuer Analytische Chemie **280**: 11-120.
- 8.2. O'Connor, B.H., Kerrigan, G.C., Thomas, W. and Gasseng, R. (1975). Analysis of the heavy element content of atmospheric particulate fractions using X-ray fluorescence spectrometry. X-ray Spectrometry **4**: 190-195.
- 8.3. Pimenta, J.A., Chan, C.C.Y. and Rees, G.S. (1977). A Rapid Method for the Determination of Lead in Suspended Air Particulate Matter - Preliminary Report. Laboratory Services Branch, Ministry of the Environment, Rexdale, Ontario.
- 8.4. Pimenta, J.A., Mace, V., Rees, G.S. and Roberts, P.J. (1978). The Determination of Optimal Parameters for the Analysis of Lead by X-ray Fluorescence Spectrometry. Laboratory Services Branch, Ministry of the Environment, Rexdale, Ontario.

THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

Method 5

X-ray Fluorescence Spectrometry

5 - 3: Ca, K, Cl, S, P, Si in Vegetation

SUMMARY

Matrix.	The method is used routinely for Ca, K, Cl, S, P and Si determination in vegetation and vegetation like material.
Substance determined.	Total Ca, K, Cl, S, P and Si.
Interpretation of results.	Results are reported as % dry weight.
Principle of method.	The sample is subjected to X-ray photons from a chromium target X-ray tube. The fluorescent X-rays produced by the sample are diffracted by the analyzing crystal and are measured using an appropriate detector.
Time required for analysis.	30 minutes for 6 elements in 10 samples.
Range of application.	See Table 5.1.
Standard deviation.	See Table 5.1.
Detection criteria.	See Table 5.1.
Interferences and shortcomings.	No analytical line interferences; matrix correction is required.
Minimum volume of sample.	2 grams dried and powdered vegetation.
Preservation and sample container.	Store pelletized samples in a desiccator.
Safety considerations.	Normal safety precaution of an X-ray laboratory. Radiation monitoring devices must be worn.

TABLE 5.1
X-RAY FLUORESCENCE DATA

All values $\mu\text{g/g}$

Matrix	Element	Detection Limit	Top of Range	Precision Between Run	Accuracy*	Notes
Vegetation	Ca	.02	4.5	± 0.07	± 0.19 at 4	
	Cl	.01	3.0	± 0.01	± 0.10 at 3	
	K	.02	4.0	± 0.04	± 0.12 at 4	
	P	.02	0.6	± 0.02	± 0.03 at .6	
	S	.02	4.0	± 0.02	± 0.50 at 4	
	Si	.03	2.0	± 0.03	± 0.10 at 2	

* NBS Orchard Leaves

THE DETERMINATION OF TRACE METALS

By Atomic Spectroscopy

Method 5

X-ray Fluorescence Spectrometry

5 - 3: The Analysis of Plant Material

1. Introduction

The matrix elements in vegetation are determined by allowing primary X-rays from an X-ray tube to impinge upon the surface of the vegetation pellets. The resulting fluorescence radiation (secondary X-rays) from the sample is collimated, directed to an analyzing crystal and diffracted at various angles. A detector placed at the correct angular location for each element measures the intensity of the emitted radiation. The intensity is proportional to concentration if a small correction is made for the matrix effect.

2. Interference and Shortcomings

There are two types of interferences generally encountered in X-ray analysis. Enhancement of the radiation by overlapping lines and absorption/enhancement of the radiation by major elements present in the sample. The first can be overcome by using an interference free line for measurement, or by mathematical correction. In the second case, the concentration of the other major elements present in the vegetation sample (Ca, K, Cl, S, P, Si) must be estimated so that a matrix correction can be applied to the results. The process is not time consuming, if the necessary calculations are performed by means of a desk-top calculator. The correction for most vegetation samples is small.

Insufficiently ground samples may give false readings due to the increase in scattered radiation from the front surface of the pellet.

3. Apparatus

- 3.1. Three place analytical balance (top loading).
- 3.2. Hydraulic press, Herzog HTP 40 or equivalent.
- 3.3. 20 mm diameter die.
- 3.4. Siemens SRS 1 X-ray Fluorescence Spectrometer with logic controller or equivalent.
- 3.5. Plastic vials with snap cap, 7 gram capacity.

4. Reagents

- 4.1. Hoechst "C" wax (microcrystalline powder), obtainable from Hoechst Canada Ltd., Toronto.

5. Procedure

5.1. Standardization

The procedure for standardization is lengthy. Rather than detail the procedure here, the reader is directed to reference 8.3 of the bibliography where the procedure is outlined in detail. A summary follows.

- 5.1.1. The standardization of the X-ray requires that a large number of classically analyzed vegetation samples be available.
- 5.1.2. The samples are inserted into the sample chamber of the X-ray spectrometer and the count rate for the 6 elements is obtained using the conditions described in Table 5.2.
- 5.1.3. Matrix correction procedures are applied to each of the 6 elements in turn and calibration curves are obtained by plotting net intensity vs. the matrix corrected concentration of the calibration element.
- 5.1.4. At the time of calibration, Quality Control and Daily Standardization Pellets were analyzed. (See below).

5.2. Daily Standardization and Quality Control Pellets

- 5.2.1. In order to check the instrument stability from day to day and to correct for long term drift of the instrument, four "Daily Standardization Pellets" are used.
- 5.2.2. Weigh out in duplicate 2.00 g of N.B.S. tomato leaves and (in duplicate) 2.00 g of N.B.S. spinach leaves into 4 new clean plastic vials.
- 5.2.3. Add 0.50 g of wax to each vial.
- 5.2.4. Agitate, transfer the powder to the die and pelletize at 25 tons for 30 seconds.
- 5.2.5. Label the pellets and store in a desiccator.
- 5.2.6. Prepare an N.B.S. orchard leaf Quality Control Pellet (A) and an N.B.S. tornato leaf Q.C. Pellet (B) in the same fashion.

5.3. Sample Analysis

- 5.3.1. Weigh out 2.00 g of vegetation sample into a new clean plastic vial.
- 5.3.2. Add 0.50 g wax to the vial.
- 5.3.3. Cap the vial and mix by hand until the vial contents are homogeneous.

- 5.3.4. Transfer the powder to the die, level the surface of the mixed powder and pelletize. A pressure of 25 tons for 30 seconds is sufficient to give a sturdy pellet.
- 5.3.5. Label the pellet, using a gummed label on the non-smooth side, taking care not to contaminate the smooth surface during handling. Store pellets in a desiccator prior to analysis.
- 5.3.6. Load the sample changer with the 4 Daily Standardization Pellets, the N.B.S. Orchard Leaves QC (A), the N.B.S. tomato leaves QC (B).
- 5.3.7. Measure the fluorescence of Ca, K, P, Cl, S, Si according to the operating instruction of the instrument and using the parameters shown in Table 5.2.
- 5.3.8. Calculate the daily standardization factor (f_{α}) for each of the six elements (α). The value of f_{α} is obtained from:

$$f_{\alpha} = C_{\alpha}^0 / C_{\alpha}$$
 where C_{α}^0 is the number of counts recorded at the time the calibration was developed and C_{α} is the number of counts observed from the present run.

$$\alpha = \text{Ca} \dots \text{Si}$$
- 5.3.9. If $0.9 \leq f_{\alpha} \leq 1.1$ then proceed to 5.3.10, otherwise restandardize the instrument.
- 5.3.10. Insert the vegetation samples into the spectrometer sample changer and follow step 5.3.7.

6. Calculation and Reporting

- 6.1. The calibration curves obtained from the standardization procedure of section 5.1 are used to calculate the non-matrix corrected concentration (C_{α}^*) for the 6 elements. The equation used is of the form

$$(C_{\alpha}^*) = (Rf_{\alpha} - b)/a$$

Where:

R is the number of XRF counts.

f_{α} is the daily standardization factor (defined above).

b is the intercept of the calibration line.

a is the slope of the calibration line.

- 6.2. The non-matrix corrected concentrations are corrected for matrix effects using the techniques described in reference 8.3 of the bibliography.
- 6.3. The necessary calculations for the completion of step 6.2 are performed by an HP9825 microcomputer. The final results are reported to two significant digits as a % dry weight of vegetation.

7. Precision and Accuracy

Varies from element to element. See Table 5.1.

8. Bibliography

- 8.1. K. Norrish and J.T. Hutton, Plant Analysis by X-ray Spectrometry: Low Atomic Number Elements, Sodium to Calcium. X-ray Spectrometry, 6(1), 6-II (1977).
- 8.2. J. A. Pimenta. Vegetation analysis by X-ray Fluorescence Part I: Sulfur, Chlorine, Phosphorus. Ontario Ministry of the Environment 1972.
- 8.3. R. H. Judge and J.A. Pimenta. The Analysis of Plant Material by X-ray Fluorescence Spectrometry Part I: Chlorine, Sulfur, Phosphorous, Calcium, Potassium, Silicon. Ontario Ministry of the Environment 1979.

TABLE 5.2

INSTRUMENTAL PARAMETERS OF THE XRF SPECTROMETER

Element	Angle (2θ)	Detector	Pulse Height Analyzer Setting		Collimator (mm)	Counting time (sec)	Crystal	Order
			<u>Baseline</u>	<u>Width</u>				
Ca	100.325	F /C	1.78	2.83	0.4	4	PET	2
K	117.660	F /C	1.78	2.83	0.4	4	PET	2
P	133.130	F /C	1.78	2.83	0.4	4	GRAPHITE	1
Cl	65.340	F /C	1.78	2.83	0.4	4	PET	1
S	106.330	F /C	1.78	2.83	0.4	4	GRAPHITE	1
Si	109.105	F /C	1.78	2.83	0.4	4	PET	1

Power 45 kV, 30 mA

F/C Flow Proportional Counter

PET Pentaerythritol crystal

THE DETERMINATION OF MOISTURE CONTENT

The moisture content may be determined on solid or semi-solid samples such as soils, sediments and sludges. On soil and sediment samples, the percent moisture is required for the conversion of results reported as percent dry weight to percent wet weight. For sludge samples the test is performed as a routine process control at the treatment plant.

The test result, while traditionally reported as percent moisture, is in fact a measurement of all material in the sample which is volatile at 103°C. The result reported may be frequently be higher than the actual moisture content of the sample.

Sample Handling and Preservation

Sediment samples should be collected in wide-mouth glass bottles with moisture-tight closures. Bottles should not be filled to more than 70% capacity to allow space for possible gas generation. Soil samples may be collected in glass pomade jars with moisture tight caps.

Samples should be stored under refrigeration and tested within 2 or 3 days from the time of collection. This is particularly important with sludges, due to their high bacterial activity.

Selection of Method

The method used in this laboratory is an indirect gravimetric method. Several other methods are also available (8.2, 8.3). A method employing a conductivity measurement after the addition of isopropanol and sodium chloride is used for soils, sediments and sludges prior to extraction of the samples for PCB and OC determination. This method is outlined in step 5.7 under the Determination of Organochlorine Pesticides and Polychlorinated Biphenyls.

MOISTURE CONTENT

Indirect Gravimetric Method

SUMMARY

Matrix.	This method is used for moisture determinations on sludge, soil and sediment samples.
Substance determined.	Moisture (water).
Interpretation of results.	Results are reported as percent by weight of the sample. The test detects the loss of that fraction of the water content which will evaporate in 16 hours at 103°C and also any other constituents which volatilize under the same conditions.
Principle of method.	A weighed sample is heated to drive off moisture and the weight loss on heating is reported as a percentage of the initial weight taken.
Time required for analysis.	The analysis requires 20 hours and up to 30 samples may be analyzed in a batch.
Range of application.	This test is suitable only for solid and semi-solid samples.
Standard deviation.	Based on 18 samples of sediments analyzed in duplicate the relative standard deviation was 0.7% of the reported value in the 15 to 45% moisture range.
Accuracy.	It is recommended that analyses be performed in duplicate to ensure that representative aliquots of the sample have been taken.
Detection criteria.	Not applicable.
Interferences and shortcomings.	Other volatile materials in the sample may be measured along with the moisture. Since they will be reported as moisture, this will lead to high results. In sludges, it is difficult to determine the point at which the sample is completely dry since there is a continuing weight loss due to decomposing organic materials.
Minimum volume of sample.	10 g of sample is required for a single determination.
Preservation and sample container.	Wide mouth glass bottles with polyvinylidene chloride lined closures are preferable. Samples should be stored at low temperatures to minimize bacterial activity. Soils may be collected and stored in pomade jars with air tight tops.

**Safety
considerations.**

Sludge samples should not be shaken since they may be under pressure due to gas generation. Bottles which are more than 70% filled are discarded without opening since they represent a hazard to the analyst.

MOISTURE CONTENT

Indirect Gravimetric Method

1. Introduction

Percent moisture is determined as that percent weight fraction of the sample which is lost after heating overnight at 103°C.

2. Interferences and Shortcomings

High results may be obtained if the sample is incompletely dried, allowed to lose moisture before weighing or allowed to absorb moisture after initial weighing. The temperature and duration of drying also plays an important role since the water of crystallization of many compounds will not be expelled, and some substances may be altered by chemical reaction and the volatile portions expelled. For a discussion of the latter interferences consult Sokoloff, (1933) (8.4).

3. Apparatus

- 3.1. Drying oven, vented, convection type, capable of maintaining a temperature of 103° - 105°C.
- 3.2. Analytical balance, capable of weighing to 0.01 g.
- 3.3. Evaporating dish, porcelain, 50 ml capacity.
- 3.4. Desiccator.
- 3.5. Spatula.

4. Reagents

- 4.1. Sodium lauryl sulphate ($C_{12}H_{25}OSO_3 \cdot Na$), powder, cleaning solution for washing dishes.

5. Procedure

- 5.1. Clean an evaporating dish and dry it for two hours at 100°C. Allow the dish to cool in a desiccator. Weigh the dish and record the weight to nearest 0.01 g.
- 5.2. Transfer 5 - 10 g of sample into a pre-weighed dish. Solid samples may first have to be broken up with a spatula in order to obtain a representative aliquot. Semi-solid samples must be stirred with a spatula prior to sampling in order to homogenize them.

NOTE: Do not shake sludge samples.

- 5.3. Weigh the dish and sample immediately to avoid loss of moisture.
- 5.4. Record weight of dish plus wet sample to nearest 0.01 g.
- 5.5. Place the dish plus wet sample in the oven at 103°C for 16 hours.
- 5.6. Remove the dish from oven and place in a desiccator for 2 hours.
- 5.7. Remove the dish plus dried sample from the desiccator and weigh immediately to avoid moisture absorption.
- 5.8. Record the weight of dish plus dried sample to nearest 0.01 g.

6. Calculation and Reporting

wet weight of aliquot (g) = (d + ws) - d

dry weight of aliquot (g) = (d + ds) - d

% moisture in sample = $\frac{ws - ds}{ws} \times 100\%$

Where:

d = weight of dish (g)

ws = wet weight of sample (g)

ds = dry weight of sample (g)

Report the results according to the following schedule:

Range	Report to
0.1 - 9.9%	2 significant figures
10.0 - 100%	3 significant figures

7. Precision and Accuracy

Based on duplicate analyses of 18 sediment samples, a relative standard deviation of 0.7% was obtained in the 15 to 45% moisture range.

8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater. 13th edition, Section 224G, American Public Health Association, Washington, D.C., 535-541.
- 8.2. Kolthoff, I.M. and Sandell, E.B. (1969). Quantitative Chemical Analysis. 4th edition. MacMillan, New York, 192-196.
- 8.3. Leley, V.K., Sawarkar, N.J. and Badwal, L.K., (1971). An electrometric method for the determination of soil moisture. Analyst **96**: 460-462.
- 8.4. Sokoloff, V.P. (1933). Water of crystallization in total solids of water analysis. Industrial Engineering Chemistry, Analytical Edition, **5**: 336.

THE DETERMINATION OF AMMONIA NITROGEN

Ammonia nitrogen is usually present in domestic wastewaters, and often found in industrial effluents. Moreover, biochemical reduction may produce ammonia in groundwaters. The ratio of free ammonia (NH_3) to ammonium ions (NH_4^+) is dependant upon the water's pH value and temperature. Ammonia nitrogen may also be present in the form of a metallic ion complex.

In the NH_3 form, ammonia nitrogen is particularly toxic to fish, and all forms exert a high oxygen demand during conversion to nitrite/nitrate. Ammonia nitrogen interferes with water treatment procedures by reacting with chlorine to form chloramines.

Ammonia data are useful as measures of water quality, and in interpreting the effectiveness of the waste stabilization process at sewage treatment plants.

Sample Handling and Preservation

Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

At room temperature bacteria can convert organic nitrogen to ammonia, and ammonia to nitrite. These reactions are accelerated for domestic wastes due to the abundance of bacteria.

Selection of Method

Basically, ammonia nitrogen is determined by phenol-hypochlorite colorimetry, but variations are introduced by individual laboratories due to differences in sample types and data use. The following table indicates current practice.

Sample Type	Pretreatment	Overall Analytical Range (mg/ as N)	Dual Range	Automated Blanking
Air Filters	elution with DDW	0.007-2.00	Yes	Yes
Precipitation	none	0.007-2.00	Yes	Yes
Rivers and lakes*	manual filtration (glass fibre)	0.007-2.00	Yes	Yes
Sewage, drinking waters, leachates and industrial wastes**	automated filtration (paper)	0.2-50.0	Yes	No
Anaerobic sludges***	centrifuge plus automated distillation of supernatant	10-1000	No	No

* Method A, ** Variation # 1 of Method A, *** Variation # 2 of Method A

AMMONIA NITROGEN

Automated Phenate-Hypochlorite Method A

SUMMARY

Matrix.	Method A is routine procedure for clean rivers, lakes, and precipitation samples.
Substance determined.	Ammonia (NH_3), ammonium ion (NH_4^+), plus chloramines (NH_2Cl , NHCl_2 , NCl_3).
Interpretation of results.	The results are reported in mg /l as N. Occurrence is widespread; however, a concentration greater than 1 mg /l as N in surface waters is often indicative of sanitary pollution. The ammonia (NH_3) fraction can be calculated from the result reported, the temperature and the pH of the sample.
Principle of method.	Ammonia is determined as one of four automated nutrient tests performed simultaneously on the same aliquot of filtered sample. The procedure which is based on the formation of an indophenol blue product in buffered alkaline media, includes a blanking system to compensate for the sample matrix. In the color formation stream, ammonia is converted to indophenol blue using sodium nitroprusside as catalyst. The blanking stream differs from the color formation stream in only one respect: the flow of the catalyst reagent is replaced by an equal flow of distilled, deionized water. Two analytical ranges are obtained from the output signal of the colorimeter.
Time required for analysis.	Approximately 24 analyses can be performed in an hour.
Range of application.	Dual ranges on undiluted sample: 0.007 - 0.400 mg/l as N and 0.400 - 2.00 mg/l as N. Higher levels are determined by dilution of the filtered sample.
Standard deviation.	Based on data for duplicate analyses, the average standard deviations for the low and high analytical ranges are 0.0046 and 0.0139 mg/l as N respectively.
Accuracy.	Average recovery of four quality control solutions is 101% with relative standard deviations ranging from 1.5 to 3.2%.
Detection criteria.	0.0071 mg/l nitrogen.

Interferences and shortcomings.

High levels of heavy metals, natural color constituents, and short-chain organic amines are interferences; for such samples an automated distillation procedure should be employed.

Minimum volume of sample.

75 ml.

Preservation and sample container.

Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

Safety considerations.

NOTE: Phenol is an acid, highly toxic and readily absorbed through the skin. In case of contact, wash the affected area continuously with water and soap (or mild detergent) for a least five minutes.

AMMONIA NITROGEN

Automated Phenate-Hypochlorite Method A

1. Introduction

This procedure is designed for relatively clean rivers, lakes and precipitation samples.

A filtered portion of the sample is presented to the AutoAnalyzer where, as one of four concurrent tests, a proportioned aliquot is withdrawn into the ammonia manifold. For the color formation stream, a phosphate buffer, alkaline phenate reagent, commercial chlorine bleach, and sodium nitroprusside are proportioned in turn into the sample stream which is already segmented with air. The reaction rate is increased and stabilized by elevating the temperature to 37.5°C. The blanking stream differs from the color formation stream in only one respect: the nitroprusside catalyst is replaced by an equal flow of DDW. The absorbance of the blue indophenol species (which is formed in proportion to the concentration of ammonia in the sample stream) is measured colorimetrically at 630 nm using 5 cm flow cells. Two analytical ranges are obtained from the output of the AAI colorimeter. The result in mg/l nitrogen is read from the appropriate chart recorder trace by comparison with peaks produced by similarly treated standards (8.1., 8.2.).

2. Interferences and Shortcomings

High levels of heavy metals, natural color constituents, and short-chain organic amines are interferences. Such samples should be pretreated by distillation (8.3.).

3. Apparatus

- 3.1. Filtration apparatus constructed as shown in Figure 1.
- 3.2. Glass fibre filters, Reeve Angel 934AH, 4.25 cm diameter. (Experience indicates this paper to be free of contamination.)
- 3.3. Automated sampler.
- 3.4. Proportioning pump.
- 3.5. 2 heating baths (38°C) with 7.7 ml delay coils.
- 3.6. AutoAnalyzer II colorimeter equipped with 630 nm filters and 5 cm flow cells; electronic signal from colorimeter is split to obtain two analytical ranges.
- 3.7. Voltage regulator
- 3.8. Chart recorder (2-pen).

3.9. Pump tubing and associated glassware as indicated in Figure 2.

3.10. Culture tubes: 18 x 150 mm.

3.11. Culture tube racks of 40 tube capacity.

3.12. Dilution tubes, 50 ml capacity.

4. Reagents

4.1. Ammonium chloride, NH_4Cl ; reagent grade powder.

4.2. Phenol, $\text{C}_6\text{H}_5\text{OH}$; reagent grade crystals, 500 g bottles.

4.3. Sodium hypochlorite solution, household chlorine bleach, 5.25% available chlorine.

4.4. Sodium hydroxide, NaOH ; reagent grade pellets, 5 lb bottles.

4.5. Ethylenediaminetetra-acetic acid, disodium salt, dihydrate (EDTA), $(\text{CH}_2\text{N}(\text{CH}_2\text{COOH})\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$; reagent grade powder.

4.6. Potassium phosphate, dibasic, K_2HPO_4 ; reagent grade powder.

4.7. Potassium ferrocyanide, trihydrate, $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$; reagent grade crystals.

4.8. Sodium nitroferrocyanide, dihydrate, $\text{Na}_2\text{Fe}(\text{CN})_6 \cdot 2\text{H}_2\text{O}$; reagent grade crystals.

4.9. Stock Phenol Solution

Melt 500 g of reagent grade phenol in its original container by warming in a water bath. Add 35 ml of distilled, deionized water to the liquified phenol and allow to cool. Store this liquified phenol in the dark in a tightly stoppered bottle.

NOTE: PHENOL CAUSES SEVERE BURNS WITHOUT CAUSING ANY IMMEDIATE PAIN. DO NOT UNDERESTIMATE THIS DANGER. MAINTAIN A QUANTITY OF METHANOL IN THE WORK AREA AS A RINSING AGENT FOR PHENOL SPILLS. IN CASE OF FACIAL CONTACT USE WATER ONLY.

4.10. Buffer

Dissolve 167 g K_2HPO_4 and 18.6 g of EDTA in approximately 600 ml of distilled, deionized water; add 40.4 g NaOH and dilute to one liter. Reagent is stable for at least one month. Reagent $\text{pH} \approx 12.3$.

NOTE: EXTREME CARE MUST BE TAKEN IN HANDLING SODIUM HYDROXIDE, A VERY CAUSTIC CHEMICAL.

NOTE: Although the chemical equivalent of sodium phosphate may be used it is difficult to dissolve and may precipitate with minor changes in temperature.

4.12. Phenate - Reducing Agent

Dissolve 4.0 g $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ and 25 g NaOH in about 800 ml of distilled, deionized water. Add 50 ml of stock phenol solution and dilute to 1 liter.

Solution is unstable but can be used for three consecutive days. Reagent pH \approx 12.3.

4.13. Hypochlorite

Dilute 70 ml of sodium hypochlorite solution (household bleach) to 1 liter with distilled, deionized water.

4.14. Sodium Nitroferricyanide (0.4 g/l)

Dissolve 0.4 g of sodium nitroferricyanide (commonly called sodium nitropruside) in one liter of distilled, deionized water. Solution can be used for three consecutive days. Reagent pH \approx 6.5, i.e., very similar to the pH of the day's water supply.

4.15. Concentrated Stock Standard Ammonia Solution

Dissolve 3.0552 g of reagent grade ammonium chloride in ammonia free distilled, deionized water in a 1 liter volumetric flask. Dilute to volume with distilled, deionized water and mix well. Store in a tightly stoppered container under refrigeration. Concentration of ammonia is 800 mg/l as N.

4.16. Intermediate Stock Standard Ammonia Solution

Transfer a 20.0 ml aliquot of the concentrated stock standard ammonia solution to a 1 liter volumetric flask and dilute to volume with distilled, deionized water. Concentration of ammonia is 16 mg/l as N. This standard may be used for one working week.

NOTE: In actual practice one intermediate stock solution is prepared for the calibration of all four nutrient channels; thus this standard also contains nitrate: 36.0 mg/l as N, nitrite: 4.00 mg/l as N, orthophosphate: 4.00 mg/l as P.

4.17. Working Calibration Standards

Three working standards are prepared daily from the intermediate stock solution:

Low (L): dilute 5.00 ml of intermediate stock to 1.0 liter with distilled, deionized water. Concentration of ammonia is 0.080 mg/l as N.

Medium (M): dilute 20.0 ml of intermediate stock to 1.0 liter with distilled, deionized water. Concentration of ammonia is 0.320 mg/l as N.

High (H): dilute 100.0 ml of intermediate stock solution to 1.0 liter with distilled, deionized water. Concentration of ammonia is 1.60 mg/l as N.

4.18. Quality Control Solutions

For each analytical range, two quality control (QC) solutions are required. One long term blank (the water used to prepare the working QC solutions) serves both ranges. The concentrations of the QC solutions are chosen such that they cover the normal concentration range of samples being routinely analyzed. Sufficient volumes are prepared to last a minimum of 20 days of analyses; whenever new QC solutions are required, they are prepared in advance so that they may be monitored for at least three days prior to adopting them.

5. Procedure

- 5.1. Collect the samples and group them according to the bench sheets.
- 5.2. Prepare the filtration apparatus as shown in Figure 1.
- 5.3. Using only forceps, place the appropriate glass fibre filter in the funnel.
- 5.4. Fit a clean 18 x 150 mm culture tube onto the adaptor, being careful not to touch the rim or inside glass surface of the tube or the rubber adaptor.
- 5.5. Shake the sample vigorously and promptly vacuum filter two 15 ml portions, discarding the filtrate each time.
- 5.6. Collect a third portion of the filtrate for analysis. Remove the contaminated filter paper with the forceps.
- 5.7. Place the culture tube containing the filtered sample into a test tube rack in such a position that it may easily be correlated with the sample number on the bench sheet. Write the appropriate sample number on the first and last sample tube in each row.
- 5.8. Set the AutoAnalyzer into operation using cleaning, set-up and checking procedures appropriate to the manifold illustrated in Figure 2. For this procedure, the color and reference streams must reach the flow cell at the same time; the maximum permissible difference is one second when the colorimeter is operated on the Damp #1 setting. The two streams are synchronized by running an alkaline phenolphthalein solution as sample and adjusting the length of the appropriate sample line.
- 5.9. Air to the manifold is drawn through a 10% sulphuric acid wash to avoid ammonia contamination.
- 5.10. When loading the samples into the AutoAnalyzer sampler module, ensure that sample order conforms to the bench sheet.
- 5.11. Each run of samples will include all of the following units:

Set of calibration standards: H, M, L
DDW blank (day's supply): Bl
Quality Control samples: QC-A, QC-B, QC-C, QC-D
Long term blank: LTBl

The basic sample loading sequence is: 10 samples, Bl, 10 samples, L, M, H, Bl.
- 5.12. Calibrate the AutoAnalyzer system using calibration standards. Record the standard calibration setting, and check to ensure that it has not changed unduly.
- 5.13. Confirm the calibration by analyzing the quality control solutions. Record these values. For each analytical range, calculate totals and differences, e.g., QC-C plus QC-D and QC-C minus QC-D. Check to ensure that the calculated values conform to limits based on past data.

5.14. Monitor calibration standards (in-run sensitivity checks) throughout the run to determine if within-run corrections are required.

5.15. Read sample peak heights, and convert to concentration values.

6. Calculation and Reporting

If required multiply the reading by the dilution factor

$$\frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result in the answer space opposite each sample number of the bench sheet. Report the results to three significant figures if feasible.

7. Precision and Accuracy

Precision - duplicate analyses of routine samples

Range	Sample Concentrations (mg/l as N)	Standard Deviation (mg/l as N)
Low	<0.080	0.0043
	0.080 - 0.200	0.0065
	0.200 - 0.400	0.0043
High	0.400 - 1.00	0.0076
	1.00 - 2.00	0.0185

Accuracy-recovery of quality control (QC) solutions

Range	QC Concentration (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
Low	0.100	0.107	0.0032
	0.300	0.303	0.0042
High	0.50	0.489	0.0100
	1.50	1.481	0.0226

8. Bibliography

- 8.1. Crowther, Joan and Evans, John. Blanking System for the Spectrophotometric Determination of Ammonia in Surface Waters. *Analyst*, **105**, 849, 1980.
- 8.2. Harwood, J.E. and Huysen, D.J., *J. Water Res.*, **4**, 501, 1970.
- 8.3. Crowther, Joan and Evans, John. Automated Distillation - Spectrophotometry Procedure for Determining Ammonia in Water. *Analyst*, **105**, 851, 1980.

AMMONIA NITROGEN

Automated Phenate - Hypochlorite Method A

Variation #1

SUMMARY

Matrix.	Domestic water, sewage, leachates and industrial wastes.
Substance determined.	Ammonia (NH_3), ammonium (NH_4^+) and chloramines (NH_2Cl , NHCl_2 , NCl_3).
Interpretation of results.	The results are reported in mg/l as N. For effluents, ammonia nitrogen is a source of pollution to waterways. For waste treatment facilities ammonia levels provide a measure of treatment efficiency.
Principle of method.	Ammonia is determined as one of two automated nutrient tests performed simultaneously on the same aliquot of sample. A portion of this aliquot is filtered using an automated continuous filter. The filtrate is converted to an indophenol blue product in buffered alkaline media using sodium nitroprusside as catalyst. Two analytical ranges are obtained from the output signal of the colorimeter.
Time required for analysis.	Approximately 18 analyses can be performed per hour.
Range of application.	Dual ranges on undiluted samples: 0.2 - 20.0 mg/l as N and 20.0 - 50.0 mg/l as N. Higher levels are determined on diluted samples.
Standard deviation.	Based on duplicate analyses, the average standard deviations for the low and high analytical ranges are 0.19 and 0.36 mg/l as N respectively.
Accuracy.	Average recovery of three quality control solutions was 99.5% with relative standard deviations ranging from 0.8 to 2.5%.
Detection criteria.	0.19 mg/l as N.
Interferences and shortcomings.	High levels of metals are interferences. For sewage samples, the presence of bacteria may cause changes in the nitrogen balance during analysis.

**Minimum volume
of sample.**

75 ml.

**Preservation and
sample container.**

Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

**Safety
considerations.**

NOTE: Phenol is an acid, highly toxic and readily absorbed through the skin. In case of contact, wash the affected area continuously with water and soap (or mild detergent) for a least five minutes.

AMMONIA NITROGEN

Automated Phenate - Hypochlorite Method A

Variation #1

1. Introduction

The ammonia nitrogen contents of domestic water supplies, most sewages, industrial wastes, and leachates are determined using a less sensitive variation of Method A. The changes entail automated filtration, inclusion of a dilution loop, and deletion of the reference channel.

2. Interferences and Shortcomings

High levels of metals are interferences. For sewage samples, the presence of bacteria may cause changes in the nitrogen balance during analysis.

3. Apparatus (Changes)

- 3.1. Automated continuous filter instead of manual filtration unit (Figure 3). The former utilizes Schleicher and Schuell rolls of filter paper (#410).
- 3.2. Only one heating bath (38°C) with 7.7 ml delay coil is required.
- 3.3. AAll colorimeter utilizes 1.5 cm flow cells.
- 3.4. Pump tubing and glassware changes are shown in Figure 4.

4. Reagents (Changes)

- 4.1. Buffer solution is prepared by dissolving 87 g K_2HPO_4 and 37.2 g of the disodium salt of EDTA in approximately 600 ml of water; 37 g NaOH are added and the solution is diluted to one liter. Reagent pH = 11.9 to 12.0.

NOTE: EXTREME CARE MUST BE TAKEN IN HANDLING SODIUM HYDROXIDE, A VERY CAUSTIC CHEMICAL.

- 4.2. Calibration standards contain all four dissolved nutrients, prepared by diluting stock solutions of the individual components. For the working standards, the ammonia nitrogen concentrations are 40.0 mg/l as N and 16.0 mg/l as N.
- 4.3. Quality control solutions are prepared from another set of stocks and are appropriate for the applicable ranges.

5. Procedure (Changes)

- 5.1. Shake the sample vigorously and promptly fill a clean 18 x 150 mm culture tube, eliminating the manual filtration step. These samples may be covered

and refrigerated (4°C) to allow the settling of suspended matter and hence aid in preventing blockages in the analyzer system.

Analyses are completed in accordance with the standard procedure for the AutoAnalyzers commencing at step 5.8 of Method A. However, refer to Figure 3.

6. Calculation and Reporting

If required, multiply the reading by the dilution factor

$$\frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result in the answer space opposite each sample number of the bench sheet. Report the results to three significant figures if feasible.

7. Precision and Accuracy.

Precision - duplicate analyses of routine samples:

Range	Sample Concentrations (mg/l as N)	Standard Deviation (mg/l as N)
Low	<4.00	0.177
	4.00 - 10.0	0.219
	10.0 - 20.0	0.189
High	20.0 - 25.0	0.24
	25.0 - 50.0	0.43

Accuracy-recovery of quality control (QC) solutions:

Range	QC Concentration (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
Low	3.50	3.424	0.087
	14.0	13.98	0.122
High	14.0	14.01	0.158
	35.0	35.26	0.363

8. Bibliography

- 8.1. Crowther, Joan and Evans, John. Blanking System for the Spectrophotometric Determination of Ammonia in Surface Waters. Analyst, **105**, 849, 1980.
- 8.2. Harwood, J.E. and Huysen, D.J., J. Water Res., **4**, 501, 1970.
- 8.3. Crowther, Joan and Evans, John. Automated Distillation - Spectrophotometry Procedure for Determining Ammonia in Water. Analyst, **105**, 851, 1980.

AMMONIA NITROGEN

Automated Distillation Phenate - Hypochlorite Method A

Variation #2

SUMMARY

Matrix.	This method is used on anaerobic sludges.
Substance determined.	Ammonia (NH_3) ammonium (NH_4^+) and chloramines that are distilled under the experimental conditions of the distillation step.
Interpretation of results.	The results are reported in mg/l as N. The ammonia content of stabilized anaerobic sludge is utilized in estimating the value of the sludge as a fertilizer.
Principle of method.	A portion of the sludge is centrifuged at 10,000 rpm. The supernatant is then presented to the AutoAnalyzer system where it is buffered, distilled, and a portion of the vapour is condensed. The distillate is analyzed for ammonia using phenate-hypochlorite colorimetry.
Time required for analysis.	Approximately 20 analyses can be performed in one hour.
Range of application.	Range on undiluted sample is 7 to 1000 mg/l as N.
Standard deviation.	Average standard deviation for duplicate analyses is 5.66 mg/l as N.
Accuracy.	Average recovery of quality control solutions is 101.3% with a mean relative standard deviation of 2.2%.
Detection criteria.	7.44 mg/l as N.
Interferences and shortcomings.	As nitrogen balance of sludge may change between centrifuging and colorimetric measurement, analyses must be completed promptly.
Minimum volume of sample.	75 ml.
Preservation and sample container.	Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

**Safety
considerations.**

NOTE: Phenol is an acid, highly toxic and readily absorbed through the skin. In case of contact, wash the affected area continuously with water and soap (or mild detergent) for a least five minutes.

AMMONIA NITROGEN

Automated Distillation Phenate - Hypochlorite Method A

Variation #2

1. Introduction

The ammonia nitrogen contents of stabilized anaerobic sludges are determined by a variation of Method A. The changes include automated distillation of supernatant, inline dilution, and deletion of the reference channel.

2. Interferences and Shortcomings

As the nitrogen balance of a sludge may change between centrifuging and colorimetric measurement, analyses must be completed promptly.

3. Apparatus (Changes)

- 3.1. Sorval Model RC-5 Centrifuge or equivalent instead of filtration unit.
- 3.2. One distillation unit (Figure 5) and gas-liquid separator (Figure 6).
- 3.3. One heating bath (38°C) with 7.7 ml delay coil instead of two.
- 3.4. Elimination of reference channel.
- 3.5. Pump tubing and glass ware as indicated in Figure 7.

4. Reagents (Changes)

- 4.1. Disodium tetraborate, decahydrate, $\text{Na}_2\text{B}_4\text{O}_{10} \cdot 10\text{H}_2\text{O}$, reagent grade crystal.
- 4.2. Hydrochloric acid, HCl, reagent grade, concentrated.
- 4.3. Borate buffer solution is prepared by dissolving 9.50 g of disodium tetraborate decahydrate and 0.352 g of sodium hydroxide in 1 liter of distilled, deionized water. Reagent pH = 9.5.
- 4.4. Hydrochloric acid (0.1% v/v). Dilute 1.0 ml of concentrated hydrochloric acid to 1 liter of distilled, deionized water.

NOTE: EXTREME CARE MUST BE TAKEN IN HANDLING SODIUM HYDROXIDE AND CONCENTRATED HYDROCHLORIC ACID.

- 4.5. Buffer solution is prepared by dissolving 87 g K_2HPO_4 and 37.2 g of the disodium salt of EDTA in approximately 60 ml of distilled, deionized water; 37 g NaOH are added, and the solution is diluted to one liter. Reagent pH = 11.9 to 12.0.
- 4.6. Calibration standards and quality control solutions are prepared from separate stock solutions of ammonium chloride; other nutrients are not included. Concentration of ammonia in calibration standard is 800 mg/l as N. Concentrations of ammonia in quality control solutions are 700 mg/l as N and 140 mg/l as N.

5. Procedure

Samples are centrifuged at 10,000 rpm, for ten minutes and the resultant supernatant is analyzed in accordance with the standard procedure for AutoAnalyzers (see Method A and Figure 5, 6 and 7) commencing at step 5.8. Details about the operation of this distillation unit are included in reference 8.3. of Method A.

6. Calculation and Reporting

If required, multiply the reading by the dilution factor

$$\frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result in the answer space opposite each sample number of the bench sheet. Report the results to three significant figures if feasible.

7. Precision and Accuracy.

Precision - duplicate analyses of routine samples:

Sample Concentrations (mg/l as N)	Standard Deviation (mg/l as N)
<200	4.52
200 - 500	4.97
500 - 1000	5.71

Accuracy-recovery of quality control (QC) solutions:

QC Concentration (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
700	699.0	8.21
140	143.9	4.42

8. Bibliography

- 8.1. Crowther, Joan and Evans, John. Blanking System for the Spectrophotometric Determination of Ammonia in Surface Waters. *Analyst*, **105**, 849, 1980.
- 8.2. Harwood, J.E. and Huysen, D.J., *J. Water Res.*, **4**, 501, 1970.
- 8.3. Crowther, Joan and Evans, John. Automated Distillation - Spectrophotometry Procedure for Determining Ammonia in Water. *Analyst*, **105**, 851, 1980.

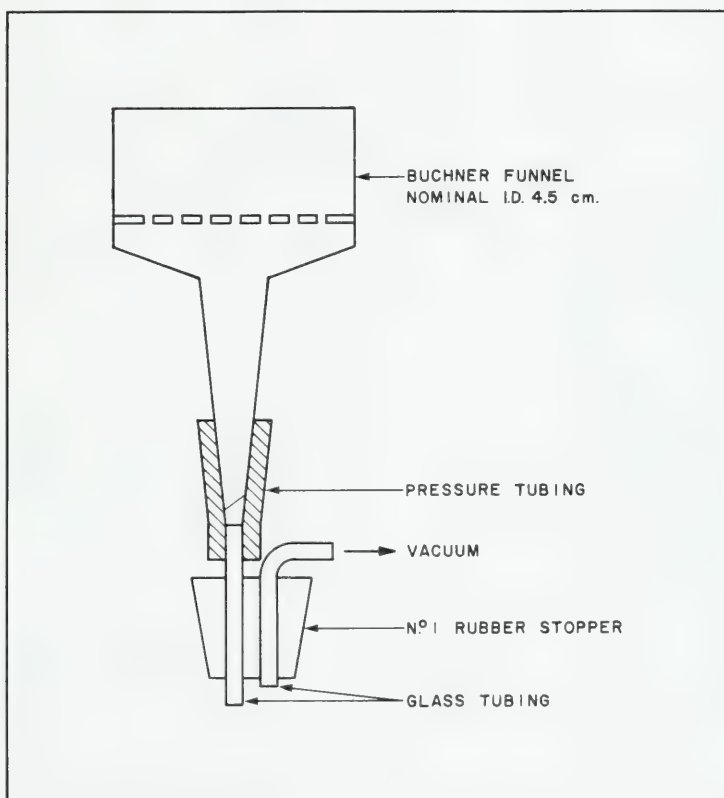


FIGURE 1 - FILTRATION APPARATUS FOR AMMONIA
NITROGEN, METHOD A.

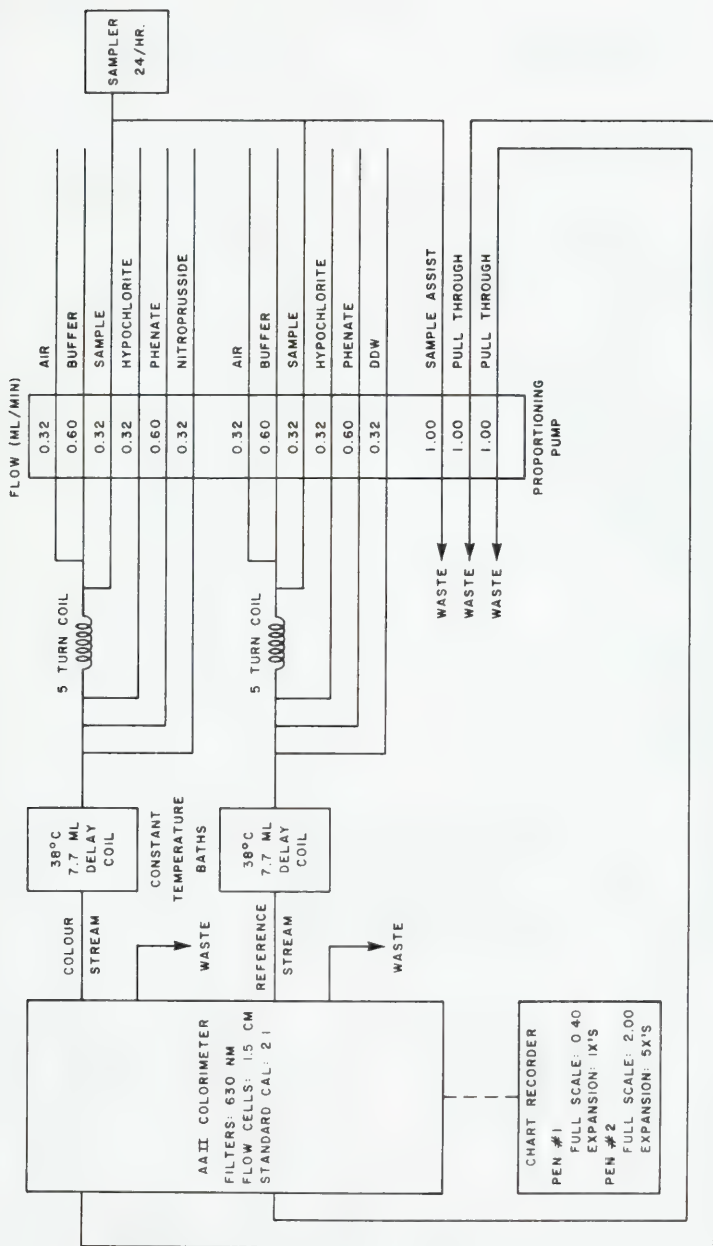


FIGURE 2 - AUTOMATED SYSTEM FOR FILTERED AMMONIA NITROGEN INCLUDING REFERENCE CHANNEL, METHOD A.

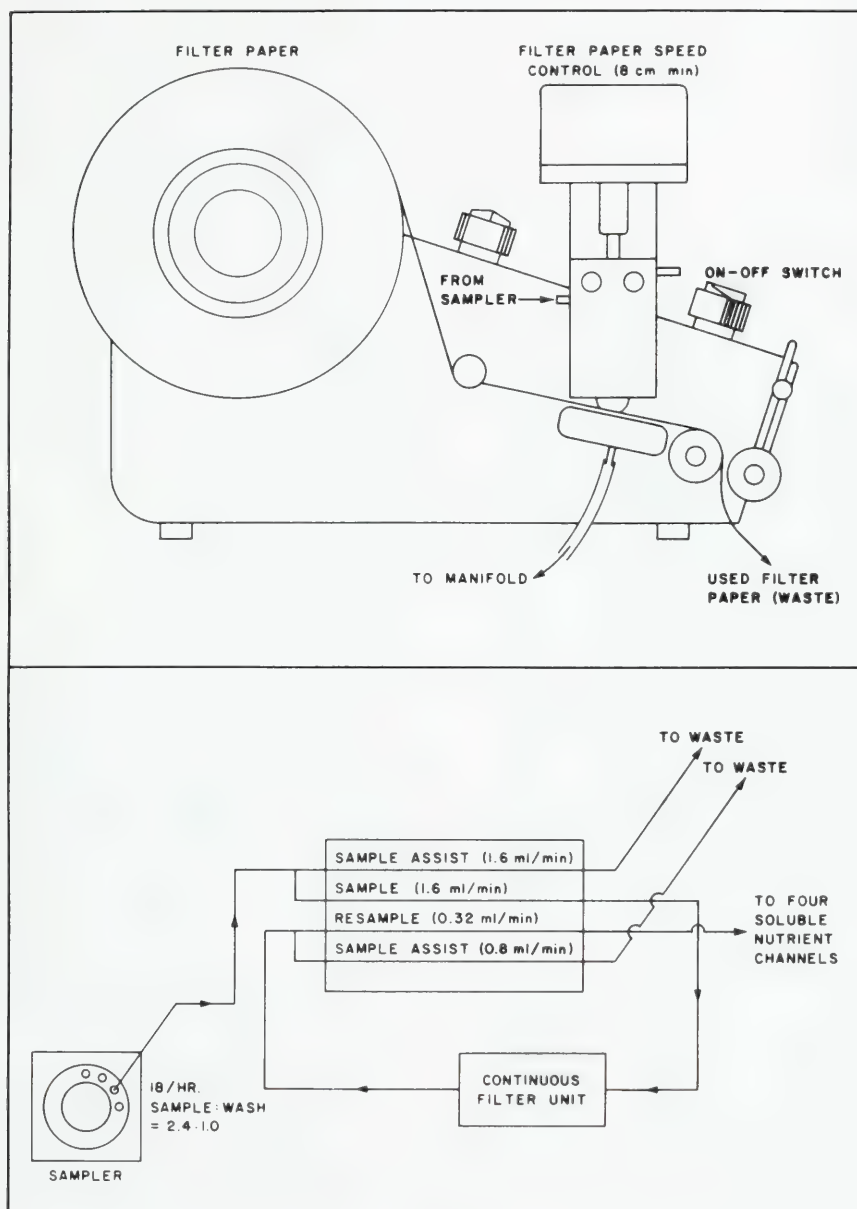


FIGURE 3 - SCHEMATIC DIAGRAM OF CONTINUOUS FILTER UNIT INCLUDING SELECTED EXPERIMENTAL CONDITIONS.

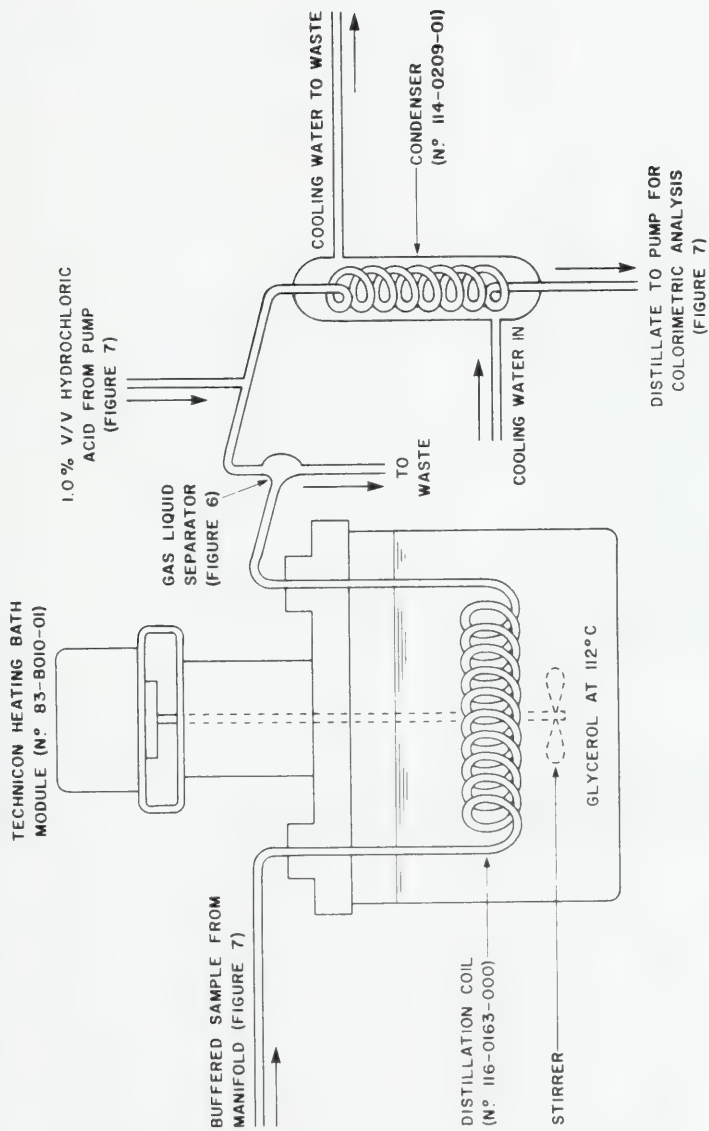


FIGURE 5 - DETAILS OF DISTILLATION UNIT FOR AMMONIA NITROGEN, VARIATION #2 OF METHOD A - SEE FIGURE 7.

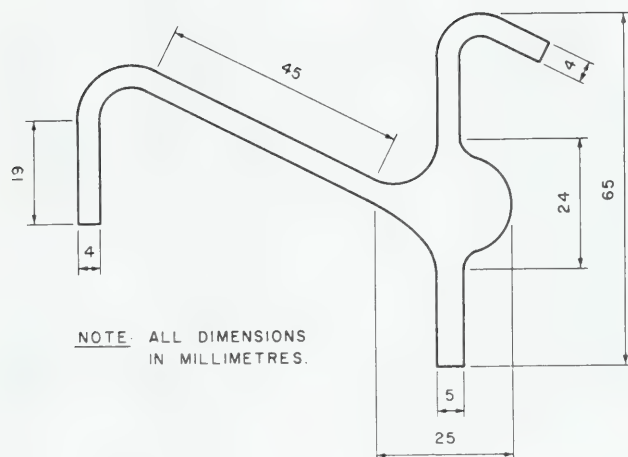


FIGURE 6 - DETAIL OF GAS-LIQUID SEPARATOR FOR AMMONIA NITROGEN, VARIATION #2 OF METHOD A - SEE FIGURE 5.

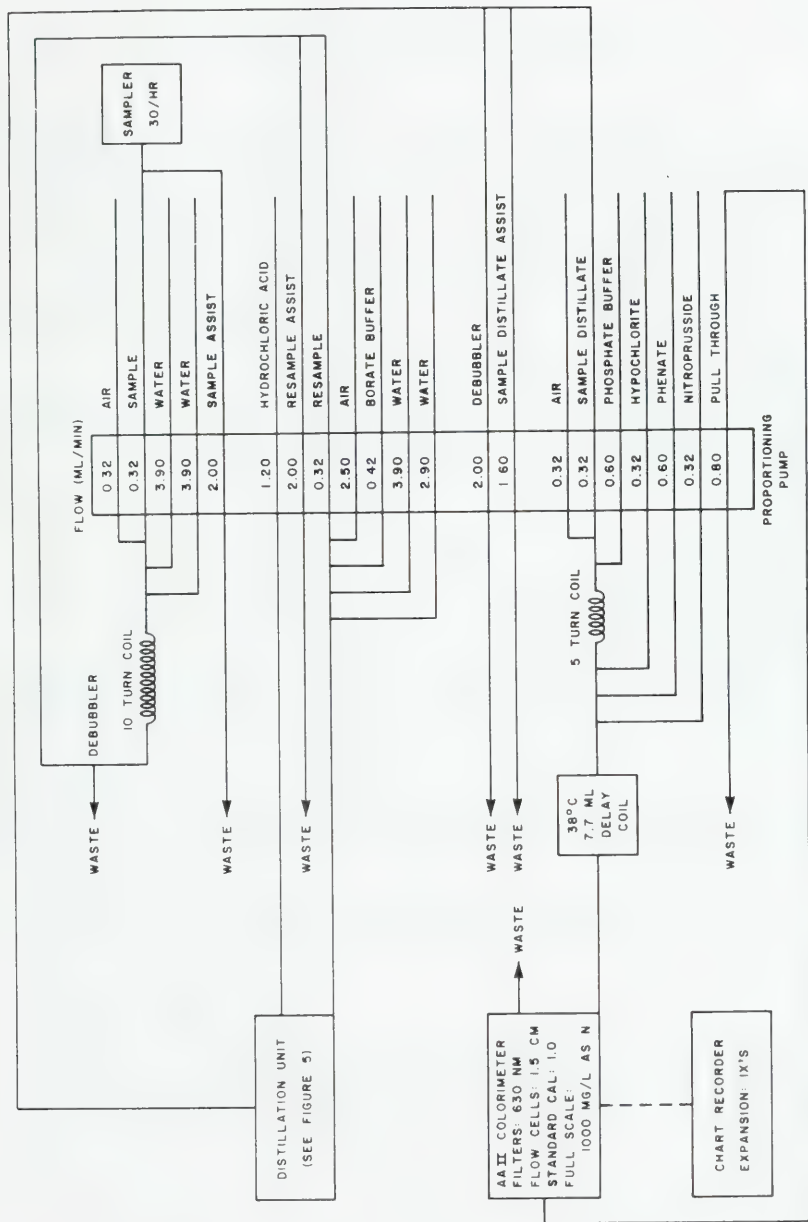


FIGURE 7 - AUTOMATED DISTILLATION - COLORIMETRIC SYSTEM FOR AMMONIA NITROGEN, VARIATION #2 OF METHOD A.

THE DETERMINATION OF NITRATE NITROGEN

Nitrates are natural constituents of plants, being present in significant quantities in many vegetables and to a lesser extent in fruits. Potential sources of nitrate in the environment arise from precipitation, the agricultural use of fertilizers, nitrogen fixation of micro-organisms and plants, decomposition of sewage wastes, and leaching from soil and rocks. Nitrates are formed via the oxidization of nitrite by autotrophic nitrifying bacteria and represent the most highly oxidized form of nitrogen within the nitrogen cycle. Surface waters generally contain trace levels of nitrate ion while groundwaters may contain significant concentrations due to soil leaching. Nitrate in water is present in the dissolved or ionic form and is potentially an alternate source of oxygen when dissolved oxygen has been depleted.

Although nitrates are an integral part of the earth's ecological system, a balance must be maintained for a healthy environment. When surface waters are well buffered due to the geologic formations of their basins, excessive inputs of nitrate accelerate eutrophication by supporting abnormally high levels of aquatic plant growth. When, however, surface waters lack natural buffering capacity, due to granite basins, the introduction of nitrates, via precipitation containing nitric acid as a pollutant, leads to acidification and the subsequent degradation of aquatic biota.

In drinking waters, excess nitrates can contribute to a disease known as infant methemoglobinemia in which the oxygen carrying capacity of the blood is inhibited. The maximum acceptable level for domestic water supplies in Ontario is 10 mg/l nitrate as nitrogen.

Waste stabilization at modern water pollution control plants normally involves the oxidation of ammonia and organic nitrogen compounds. For most of these facilities, the nitrate level in the effluent is a measure of treatment efficiency.

Sample Handling and Preservation

Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

At room temperature bacteria can convert organic nitrogen to ammonia, and ammonia to nitrite. These reactions are accelerated for domestic wastes due to the abundance of bacteria.

Selection of Method

For the precipitation samples, nitrate is determined by ion chromatography. For all other samples, nitrate is calculated, and is the difference between the combined value for nitrate plus nitrite and the value for nitrite.

NITRATE NITROGEN
Automated Ion Chromatographic Method

SUMMARY

Matrix.	The ion chromatographic procedure is used routinely for precipitation samples.
Substance determined.	Nitrate ion, NO_3^- .
Interpretation of results.	Results are reported in mg/l as N. Nitrate is the second most important anion found in acidic precipitation with sulphate being the dominant anion. The nitrate concentration of rainfall in Ontario's recreational lake country normally ranges from 0.2 - 0.7 mg/l as N.
Principle of method.	Using ion chromatography (IC), nitrate is separated from other anions in the sample using both a column packed with a proprietary ion exchange resin and an eluent composed of sodium carbonate and sodium bicarbonate. Nitrate is converted to its acid form by passage through a second ion exchange column, and then its concentration is determined by conductivity measurement.
Time required for analysis.	Approximately 7 analyses can be performed in an hour, and the equipment can be operated continuously for about ten hours.
Range of application.	Dual ranges on undiluted sample: 0.02 - 1.00 mg/l as N and 1.00 - 2.00 mg/l as N.
Standard deviation.	The average standard deviation for duplicate analyses of routine samples is 0.0148 mg/l as N.
Accuracy.	Average recovery of quality control solutions is 100% with relative standard deviations ranging from 0.46 to 1.7%.
Detection criteria.	0.021 mg/l as N.
Interferences and shortcomings.	Any anion, which has an identical retention time for the specified experimental conditions, would give a positive interference; no such anion has been identified. If the concentration of sulphate is 100X's that of nitrate - nitrogen, it is a positive interference.
Minimum volume of sample.	15 ml.

**Preservation and
sample container.**

Only polystyrene containers and polyethylene bags are recommended at this time. Samples should be shipped to the laboratory as rapidly as possible. Freezing is acceptable.

**Safety
considerations.**

Pressure developed in the IC system must not be allowed to exceed 600 psi, and the column door should be closed during analyses in case the columns shatter. Standard safety precautions are required in handling cylinders of compressed air, sodium hydroxide, and concentrated acids.

NITRATE NITROGEN

Automated Ion Chromatographic Method

1. Introduction

This procedure is designed for precipitation samples and is utilized for the simultaneous determination of chloride, nitrate, and sulphate on the same aliquot of sample. A portion of sample is fed into the AutoAnalyzer manifold where it is mixed with a solution of sodium carbonate-bicarbonate; the concentration of the latter is such that the inorganic carbon content of the stream entering the ion chromatographic (IC) module is equivalent to that of the eluent. Within the IC unit, a sodium carbonate - bicarbonate eluent in combination with a proprietary ion exchange column separates the anions according to their size and valency. The cations, associated with the eluent and sample, are converted to hydrogen ion by passage through a second ion exchange column. The conductivity of the flow from the second column is recorded continuously on a chart recorder. Carbonic acid, the acid form of the continuously flowing eluent, has a low conductivity $\approx 25 \mu S/cm$, and the latter corresponds to the baseline of the chart. As the acid form of nitrate has a high conductivity, the presence of nitrate is indicated by a peak which appears on the chart after a known period of time has elapsed. The signal from the conductivity meter is split to obtain two analytical ranges. The entire analytical operation is controlled by a microprocessor.

2. Interferences and Shortcomings

Each anionic species on the ion chromatograph is identified by its retention time; the time between sample injection and optimum peak height development. If the retention times of two anions are similar and one ion is present in considerably higher concentrations than the other, the broader and higher peak formed by the high concentration will overlap the peak of the lesser ion. Consequently, the capacity to determine an anion at low concentrations depends upon the concentrations of other ions having similar retention times. If the sulphate concentration is 100X's that of the nitrate as nitrogen, sulphate is a positive interference.

3. Apparatus

- 3.1. The IC system is schematically shown in Figure 1.
- 3.2. Automated sampler modified to accept timing signals from microprocessor.
- 3.3. Proportioning pump.
- 3.4. Ion chromatographic unit, Dionex Model 10, modified to accept signals from microprocessor and sample from manifold. (Figures 4(a) and 4(b)). Columns employed with this module are anion precolumn (0.3 x 12.5 cm), anion separation column (0.3 x 25.0 cm), and suppressor column (0.6 x 25.0 cm). The volume of the sample loop is 0.3 ml.
- 3.5. Relay Box, in-house design (Figure 2).

- 3.6. Microprocessor, Supergrator Model III. Control program is included in Figure 3, and timing sequence is detailed in Figure 5.
- 3.7. Chart recorder (2-pen).
- 3.8. Cylinder of compressed air plus reducing valve.
- 3.9. Sample tubes are polystyrene vials (2.4 x 9.0 cm) with screw caps.

4. Reagents

- 4.1. Potassium nitrate, KNO_3 , reagent grade crystals.
- 4.2. Sodium carbonate, Na_2CO_3 , reagent grade.
- 4.3. Sodium bicarbonate, NaHCO_3 , reagent grade.
- 4.4. Sodium hydroxide, NaOH , reagent grade pellets.
- 4.5. Sulphuric acid, H_2SO_4 , concentrated, reagent grade.
- 4.6. Hydrochloric acid, HCl , concentrated, reagent grade.

NOTE: All reagents and standards are prepared with distilled, deionized water and volumetric class A pipets and flasks. All glassware must be washed with 4% (V/V) HCl and thoroughly rinsed with distilled, deionized water before use.

4.7. Eluent Stock Solution

Combine 25 g sodium carbonate and 25 g sodium bicarbonate in a 1 liter volumetric flask and dilute to volume with distilled, deionized water.

4.8. Working Eluent Solution

Dilute 20.0 ml eluent stock solution to 2 liters with distilled, deionized water.

4.9. Spike Solution

Combine 5.0 g sodium carbonate and 5.0 g sodium bicarbonate in a 1 liter volumetric flask and dilute to volume with distilled, deionized water.

NOTE: Based on experience, this solution effectively eliminates loss of the baseline, due to the void volume peak, without adding a carbonate peak to the scan from the automated system (8.3.).

4.10. Regeneration Solution

Dilute 112 ml concentrated sulphuric acid to 4 liters with distilled, deionized water.

NOTE: Extreme care must be exercised in handling concentrated sulphuric acid, a most corrosive chemical.

4.11. Sodium Hydroxide Scrubber Solution

Dissolve 250 g sodium hydroxide in distilled, deionized water and dilute to 1 liter.

NOTE: Extreme care must be exercised in handling sodium hydroxide pellets and this reagent which is a very concentrated caustic solution.

NOTE: This reagent is used as a gas scrubber solution to provide air that is free of carbon dioxide to the manifold air supply and to the atmosphere over the spike solution.

4.12. Stock Standard Solution

Dissolve 2.8876 g of potassium nitrate in distilled, deionized water and dilute to 1 liter. Concentration of nitrate is 400 mg/l as N.

NOTE: In actual practice, the stock standard solution also contains chloride, 300 mg/l and sulphate, 2000 mg/l.

4.13. Intermediate Stock Standard Solution

Dilute 100.0 ml of stock standard solution to 1 liter with distilled, deionized water. Concentration of nitrate is 40 mg/l as N.

4.14. Working Calibration Standards

Six working calibration standards are prepared by diluting volumes of the intermediate stock standard solution to 1 liter with distilled, deionized water:

Designation of Standard	Volume of Intermediate Stock Standard	Nitrate Concentration
(%)	(ml)	(mg/l as N)
10	5.00	0.200
20	10.0	0.400
40	20.0	0.800
60	30.0	1.20
80	40.0	1.60
100	50.0	2.00

4.15. Quality Control Solutions

To check the calibration, two quality control (QC) solutions are analyzed daily. These solutions are prepared in the same manner as described for the calibration standards, but from a different bottle of potassium nitrate. The nitrate concentrations of the QC solutions, 0.400 mg/l as N and 1.60 mg/l as N, cover the normal concentration range of precipitation samples. Sufficient volumes are prepared to last a minimum of 20 working days. Whenever new QC solutions are required, they are monitored for at least three days prior to adopting them.

5. Procedure

- 5.1. Set up the IC system as indicated in Figure 1. System modifications and the microprocessor program are shown in Figures 2, 3, and 4. The columns that are used for the Dionex Model 10 unit are precolumn (to screen foreign matter from the sample before it reaches the expensive separator column), anion separator column (to separate nitrates from other anions in the sample), and suppressor column (to convert all cations in the sample and in the eluent to hydrogen ion).

NOTE: The operator must be familiar with the manual supplied by Dionex Corporation.

- 5.2. Fill IC reservoirs with eluent, sulphuric acid (1N), and distilled, deionized water. Be sure that the suppressor column is regenerated. Check reagent lines to ensure that no air bubbles are entrapped, and that the pump is primed.

- 5.3. Turn on IC unit, and pump eluent at 180 ml/hr (40% flow) through suppressor column (separator column is off-line). Check the system for leaks, and when response on IC conductivity meter stabilizes (between 22 and 26 $\mu\text{S/cm}$), switch the precolumn and separator columns on-line. Pressure should average 500 psi; if it exceeds 600 psi, switch the separator column off-line and find the blockage. When the problem is corrected, check for leaks again.
- 5.4. Set up 2-pen recorder and conductivity meter on IC unit; the output of the latter is one volt. Switch meter to ZERO mode, and set pens to zero lines. Switch meter to CAL mode, and set pens to 100 lines. Switch meter to LIN mode and select range of "10 μmho " as full scale. Offset needle to give a zero reading using appropriate knob on IC meter; this step compensates for the conductivity of carbonic acid. Expand the range by a factor of two for one pen of the chart recorder.
- 5.5. Turn on microprocessor (Supergrator 3), and load program 2. Set to Normal mode.

NOTE: Program 2 is retained in memory provided the microprocessor is not turned off.
- 5.6. Collect samples and group them in accordance with bench sheets.
- 5.7. Each run of samples will include all the following units:
 - set of calibration standards: 10, 20, 40, 60, 80 and 100% of full scale, i.e., 2 mg/l as N.
 - blank: day's supply of distilled, deionized water
 - quality control samples: 0.40 and 1.60 mg/l as N

The basic loading sequence is 6 samples, calibration standard, 6 samples; where the nitrate concentration of the in-run calibration standard is 0.80 mg/l as N.
- 5.8. Turn on sampler, proportioning pump, relay box, and commence run. Retention time for nitrate will vary slightly from one separator column to another, but the average time is 4.5 min and the shape of the peak is distinctive (Figure 3).
- 5.9. Check response of the quality control solutions and confirm that the results conform to the limits based on previous data.
- 5.10. When analyses have been completed, switch the separator column off-line, and wash system with distilled, deionized water until true conductivity approximately equals that of distilled, deionized water, i.e., no eluent in system. Turn off the eluent pump.
- 5.11. Regenerate the suppressor column by switching to regeneration mode, and setting timing cycles for ten minutes of regenerant and thirty minutes of distilled, deionized water. Use a flow rate of 275 ml/hr (60%), and one normal sulphuric acid as regenerant. After regeneration, the regeneration pump will turn off automatically.

6. Calculation and Reporting

- 6.1. Calibration curves for each range are prepared by plotting peak height against nitrogen concentration. If necessary, corrections are applied for in-run sensitivity changes. Nitrate concentrations are determined from the calibration curves, and results are reported to three significant figures if feasible.

7. Precision and Accuracy

Precision - duplicate analyses of routine samples

Sample Concentration Range (mg/l as N)	Standard Deviation (mg/l as N)
< 0.200	0.0126
0.200 - 0.500	0.0124
0.500 - 1.00	0.0159
1.00 - 2.00	0.0160

Accuracy - recovery of quality control (QC) solutions

QC Concentration (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
0.400	0.406	0.0067
1.60	1.60	0.0060

8. Bibliography

- 8.1. Crowther, J. and McBride, J. (1978). Analysis of Precipitation Samples by Ion Chromatography. Part 1 - Determination of Anions. Ontario Ministry of the Environment, Laboratory Services Branch, Toronto, Ontario.
- 8.2. Dionex Ion Chromatographs 10, 14, 16. Operation and Maintenance. Dionex Corporation, Sunnyvale, California.
- 8.3. McBride, J., Clarke, K., Crowther, J., Lindow, O. and Rawlings, M. W., (1979). Automation of Anion Chromatographic System. Ontario Ministry of the Environment, Laboratory Services Branch, Toronto, Ontario.
- 8.4. Crowther, J., and McBride, J., "Determination of Anions in Precipitation by Ion Chromatography", The Analyst 107, 702-709, 1981.

* CO₂ SCRUBBED AIR SUPPLIES VIA
WASH BOTTLES CONTAINING
SODIUM HYDROXIDE

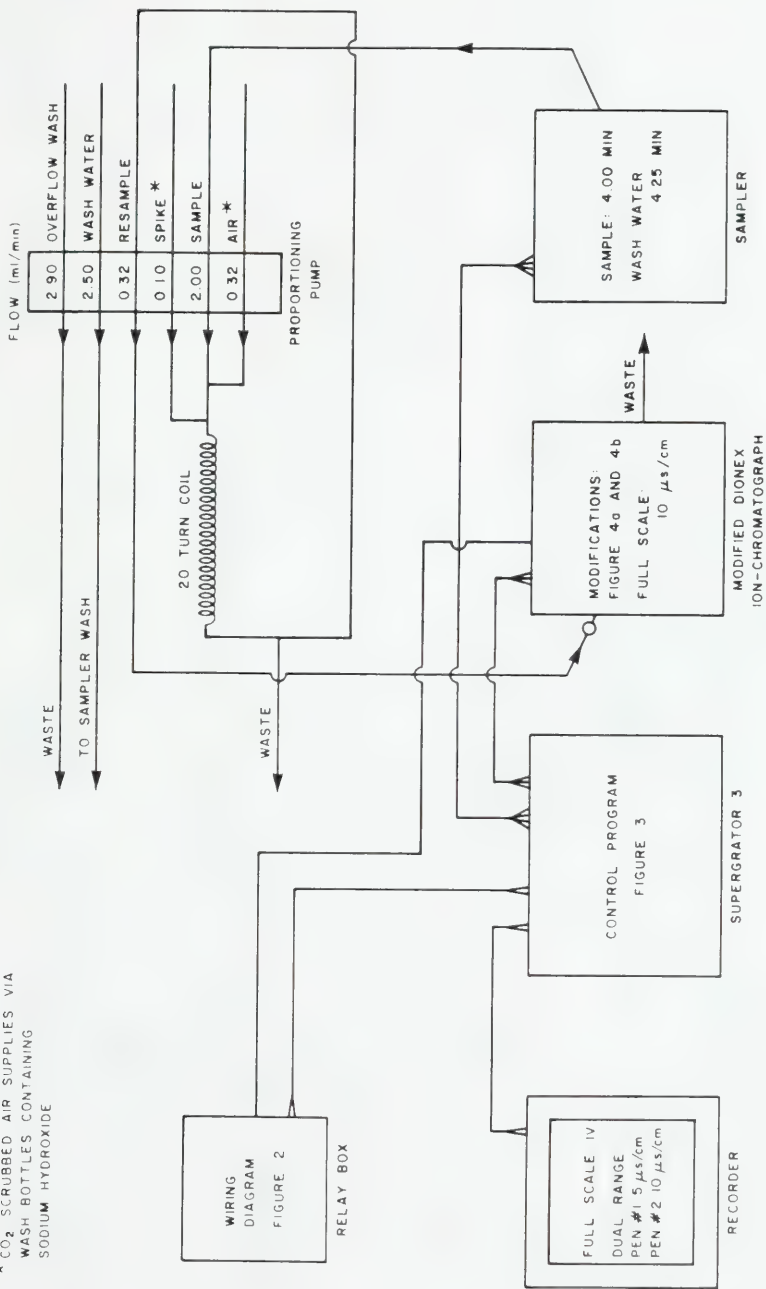


FIGURE 1 - AUTOMATED ION-CHROMATOGRAPHIC SYSTEM FOR NITRATE NITROGEN.

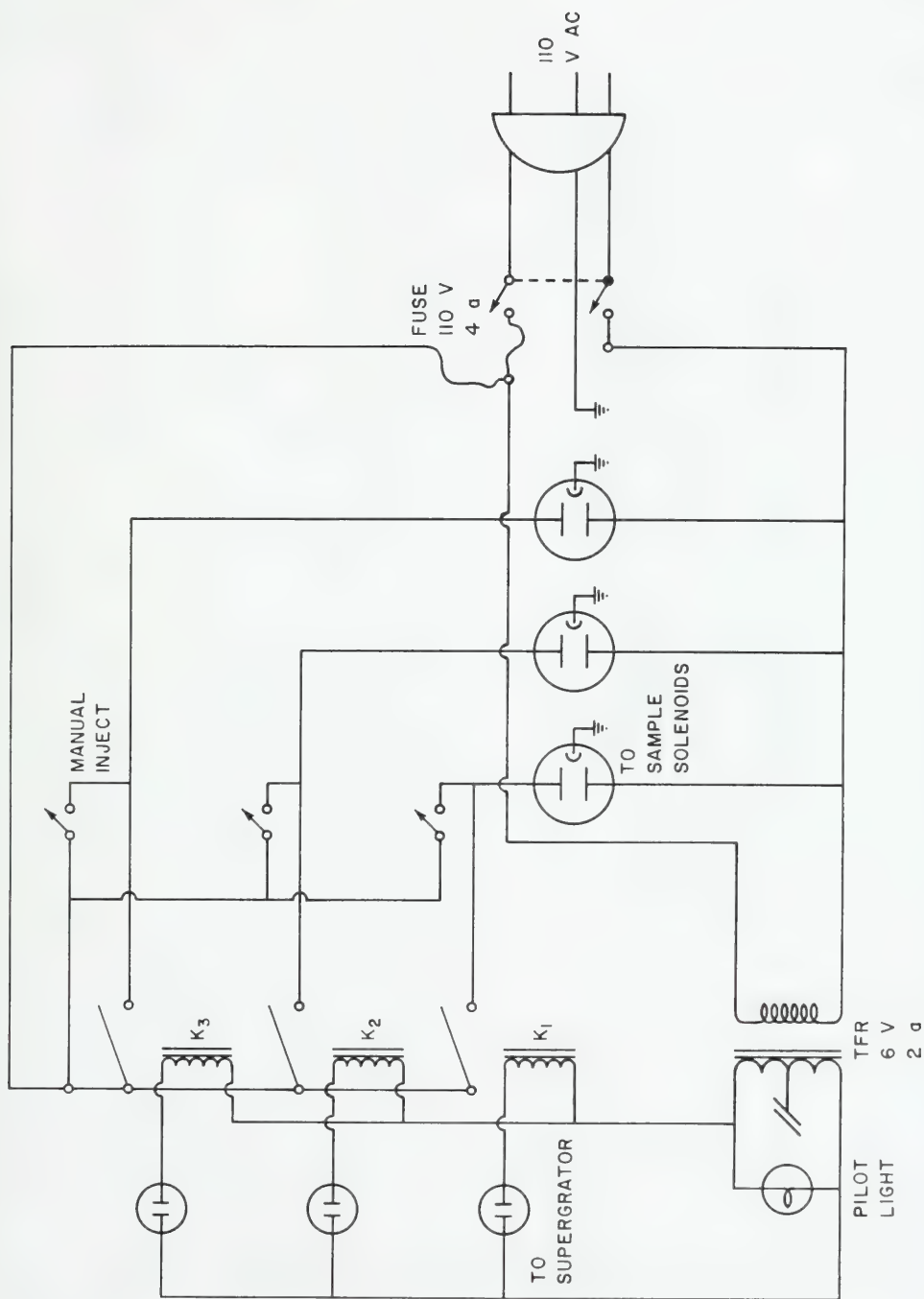


FIGURE 2 - WIRING FOR RELAY BOX.

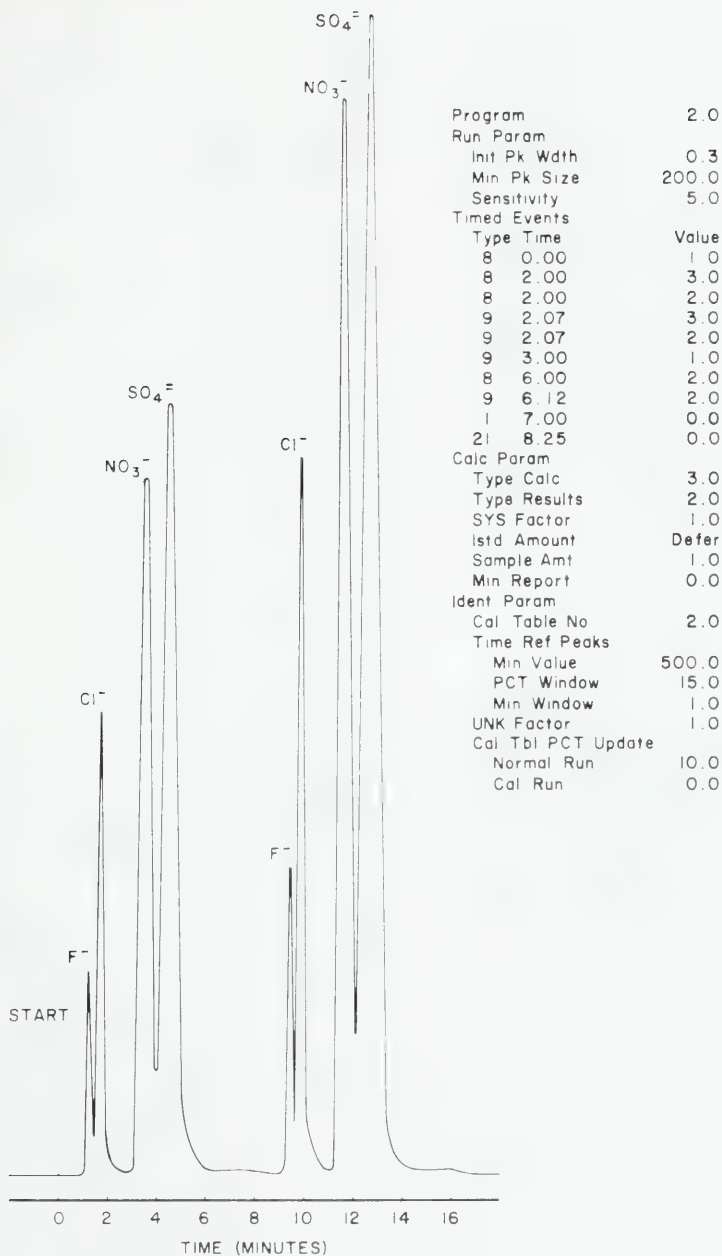
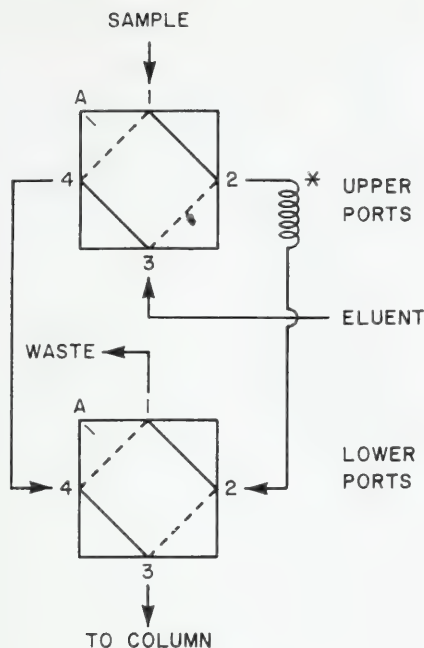


FIGURE 3 - PROGRAM 2 AND SCAN FOR DETERMINATION OF NITRATE NITROGEN BY AUTOMATED ION CHROMATOGRAPHY.



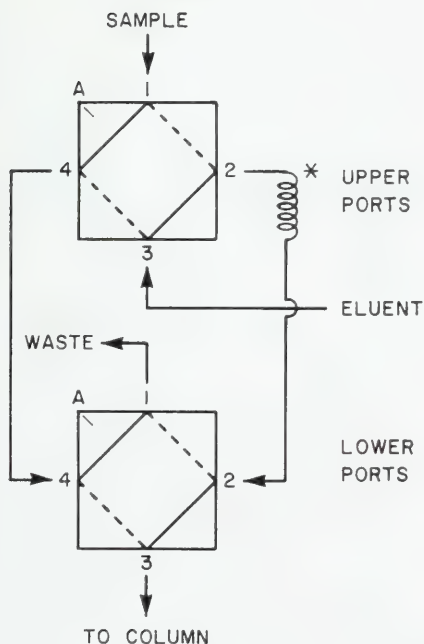
Load Position

Air on to top of valve, off to bottom, \therefore sliders in down position.

Black marker on sliders is in the A corner between ports 1 and 4.

Connection between upper and lower ports 4 is as short as possible.

Connection* between upper and lower ports 2 is of measured length and I.D. and acts as sample loop.



Inject Position

Air on to bottom of valve, off to top, \therefore sliders in up position.

All other connections, etc., are as above.

FIGURE 4(a) - SAMPLE INJECT VALVE FOR AUTOMATED I.C. SYSTEM.

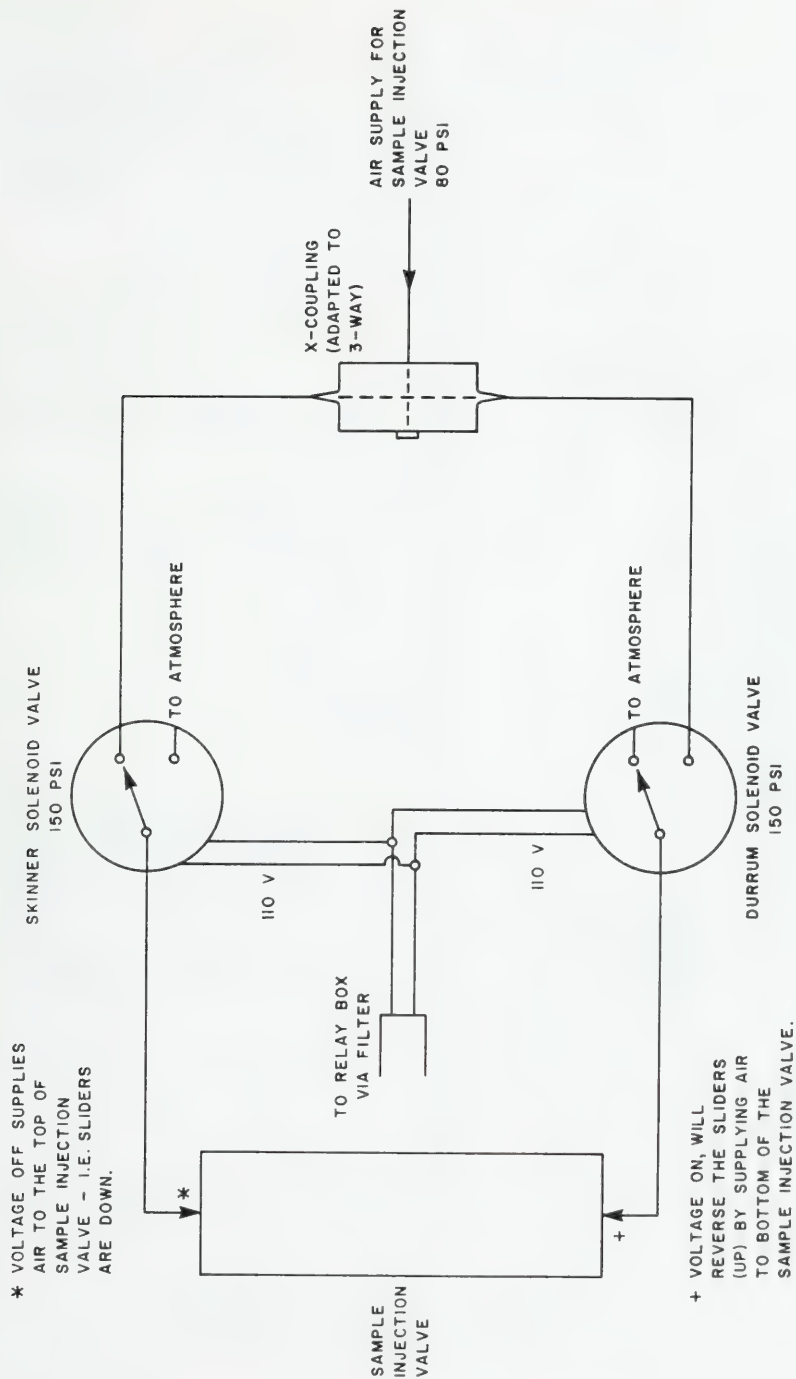


FIGURE 4(b) - WIRING AND AIR CONNECTIONS FOR SOLENOID VALVES TO OPERATE
SAMPLE INJECTION VALVE OF I.C. MODULE.

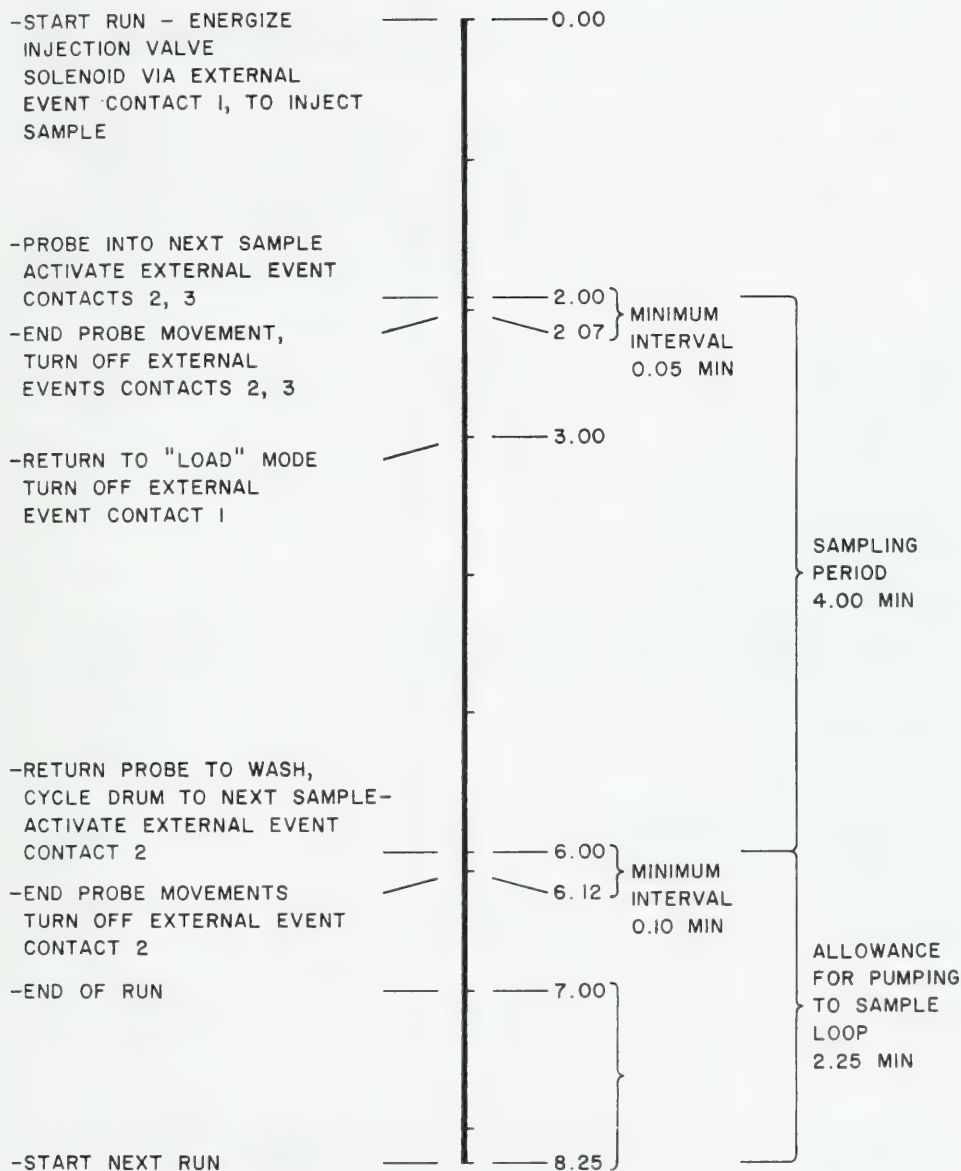


FIGURE 5 - TIMING SEQUENCE (40 % FLOW).

THE DETERMINATION OF NITRATE NITROGEN PLUS NITRITE NITROGEN

Both nitrate and nitrite anions are part of the nitrogen cycle with nitrates representing the highest state of oxidation, and nitrites being an intermediary between the formation of ammonia and the production of nitrate. In aqueous media, the concentration of nitrite is generally less than five percent of the nitrate concentration, but the proportion may be higher during some stages of treatment processes. Information about the environmental significance of these anions is found in the methodologies for nitrate nitrogen and for nitrite nitrogen.

Sample Handling and Preservation

Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

At room temperature bacteria can convert organic nitrogen to ammonia, and ammonia to nitrite. These reactions are accelerated for domestic wastes due to the abundance of bacteria.

Selection of Method

Samples are analyzed via an automated colorimetric procedure which entails converting nitrate to nitrite, and then analyzing the sample for nitrite; the original nitrite concentration is included in the result because nitrite anions are not affected by the preliminary reduction step.

The following table lists the various procedures that are available for nitrate and nitrite parameters.

PARAMETER	TYPE OF ANALYSIS	OVERALL ANALYTICAL RANGE (mg/l as N)	DUAL RANGE
Nitrate*	Ion Chromatographic	0.03 - 2.00	Yes
Nitrite	Colorimetric	0.001 - 0.100 0.02 - 2.00	No No
Nitrate plus Nitrite	Colorimetric	0.01 - 5.00** 0.1 - 50.0***	Yes Yes

* The ion chromatographic procedure is restricted to the APIOS program. For all other programs, nitrate is calculated from the values for nitrite and for nitrate plus nitrite.

** Method A,

*** Variation #1 of Method A

Hydrazine Reduction - Azo Dye Method A

SUMMARY

Matrix.	Method A is used routinely for clean rivers and lakes.
Substance determined.	Nitrate (NO_3^-) and Nitrite (NO_2^-) ions.
Interpretation of results.	Results are reported in mg/l as N. The nitrite fraction is generally less than five percent of the nitrate concentration. If water is used for drinking, the result should be less than 10 mg/l as N in order to meet the nitrate criteria.
Principle of method.	Nitrate nitrogen plus nitrite nitrogen are determined as one of four automated nutrient tests which are performed simultaneously on the same aliquot of filtered sample. Nitrate is reduced to nitrite by heating an aliquot of sample with hydrazine in alkaline media; this reaction is catalyzed by the addition of cupric ion. Subsequently an azo dye is formed in acid media by diazotizing sulphanilamide with nitrite and coupling the product with N (1-naphthyl) ethylenediamine dihydrochloride. The absorbance of the light red azo dye is measured at 520 nm and the concentration of nitrate nitrogen plus nitrite nitrogen is determined by comparison with a similarly treated series of mixed standards.
Time required for analysis.	Approximately 24 analyses can be performed in an hour and over 200 per day.
Range of application.	Dual Range: 0.01 - 1.00 mg/l as N and 1.00 - 5.00 mg/l as N. Higher concentrations are determined by dilution of the filtered sample.
Standard deviation.	Based on duplicate analyses, the average standard deviations for the low and high analytical ranges are 0.0066 and 0.0400 mg/l as N respectively.
Accuracy.	The average recovery of four standards is 101% with relative standard deviations ranging from 1.5 to 2.9%.
Detection criteria.	0.0097 mg/l N.
Interferences and shortcomings.	The pH of the sample must lie between 6 and 9. Oxidizing agents can prevent reduction of nitrate and reducing agents can reduce nitrite. Interferences from divalent cations are eliminated by the use of an ion exchange column.

Minimum volume of sample. 75 ml

Preservation and sample container. Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

At room temperature bacteria can convert organic nitrogen to ammonia, and ammonia to nitrite. These reactions are accelerated for domestic wastes due to the abundance of bacteria.

Safety considerations.

Normal safety precautions for handling strong bases and acids are required. As all naphthyl type compounds are considered potential carcinogens until proven otherwise, care should be taken in handling the powder, N (1-naphthyl) ethylenediamine dihydrochloride.

NITRATE NITROGEN PLUS NITRITE NITROGEN

Hydrazine Reduction - Azo Dye Method A

1. Introduction

This procedure is designed for relatively clean rivers and lakes.

Nitrate nitrogen plus nitrite nitrogen are determined as one of four automated nutrient tests which are performed simultaneously on the same aliquot of filtered sample. Nitrate is reduced to nitrite by heating an aliquot of sample with hydrazine in alkaline media; this reaction is catalyzed by the addition of cupric ion. Subsequently an azo dye is formed in acid media by diazotizing sulphanilamide with nitrite and coupling the product with N(1-naphthyl)ethylenediamine dihydrochloride. The absorbance of the light red azo dye is measured at 520 nm and the concentration of $\text{NO}_3\text{-N}$ plus $\text{NO}_2\text{-N}$ is determined by comparison with a similarly treated series of mixed standards.

2. Interferences and Shortcomings

The pH of the sample must lie between 6 and 9. Oxidizing agents can prevent reduction of nitrate and reducing agents can reduce nitrite. Interferences from divalent cations are eliminated by the use of an ion exchange column.

3. Apparatus

- 3.1. Filtration apparatus constructed as shown in Figure 1.
- 3.2. Glass fibre filters, Reeve Angel 934AH, 4.25 cm diameter.
- 3.3. Automated sampler.
- 3.4. Proportioning pump.
- 3.5. AutoAnalyzer II colorimeter equipped with 520 nm filters and 5.0 cm flow cells; electronic signal from colorimeter is monitored to obtain two analytical ranges.
- 3.6. Voltage regulator.
- 3.7. Chart recorder (2-pen).
- 3.8. Heating bath (38°C) with 7.7 ml delay coil.
- 3.9. Pump tubing and associated glassware as indicated in Figure 2.
- 3.10. Culture tubes, 1.8 x 15.0 cm.
- 3.11. Culture tube racks of 40 tube capacity.
- 3.12. Dilution tubes, 50 ml capacity.

3.13. Pyrex glass tube, 0.3 cm diameter x 8 cm length.

4. Reagents

- 4.1. Potassium nitrate, KNO_3 , reagent grade crystals.
- 4.2. Sodium nitrite, NaNO_2 , reagent grade crystals.
- 4.3. Sodium hydroxide, NaOH ; reagent grade pellets.
- 4.4. Hydrazine sulphate, $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$; reagent grade crystals.
- 4.5. Copper sulphate pentahydrate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; reagent grade powder.
- 4.6. Hydrochloric acid, HCl , concentrated reagent grade.
- 4.7. Sulphanilamide, $\text{H}_2\text{N}-\text{C}_6\text{H}_4\text{SO}_2\text{NH}_2$, reagent grade powder.
- 4.8. N(1-Naphthyl) ethylenediamine dihydrochloride, $\text{C}_{10}\text{H}_7\text{HNCH}_2\text{CH}_2\text{NH}_2 \cdot 2\text{HCl}$, reagent grade powder.
- 4.9. Ion exchange resin, Amberlite IR 120 (H^+), analytical grade.
- 4.10. Magnesium sulphate, heptahydrate, $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$, reagent grade crystals.
- 4.11. Calcium chloride, dihydrate, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Analar reagent grade.

4.12. Concentrated Stock Sodium Hydroxide

On a top-loading balance, weigh out 728 g of NaOH . Place approximately 1600 ml of distilled, deionized water in a 2 liter beaker and with continuous stirring cautiously add the NaOH to the beaker. When the solution is cool, dilute to 2 liters with distilled, deionized water.

CAUTION - Extreme care must be taken in handling sodium hydroxide, a very caustic chemical.

4.13. Working Sodium Hydroxide Solution

Pipet 25 ml of stock NaOH into a 1 liter volumetric flask and make up to the mark with distilled, deionized water.

EYE PROTECTION MUST BE WORN DURING THIS PROCEDURE. THIS SOLUTION IS 30% DRY WEIGHT NaOH AND MAY CAUSE SEVERE BURNS TO UNPROTECTED AREAS.

4.14. Stock Hydrazine Sulphate

Dissolve 12.00 g of hydrazine sulphate in 900 ml distilled, deionized water and dilute to 1.0 liter. The solution may be used for one month only.

CAUTION - THIS REAGENT IS TOXIC IF INGESTED.

4.15. Working Hydrazine Solution

Dilute 50.0 ml of stock hydrazine to 500.0 ml in a volumetric flask. Prepare fresh daily as the solution deteriorates and loses sensitivity after 24 hours.

4.16. Stock Copper Sulphate Solution

Dissolve 2.50 g of copper sulphate pentahydrate in distilled, deionized water and dilute to 1.0 liter.

4.17. Working Copper Sulphate Solution

Dilute 2.0 ml of stock solution to 2.0 liters with distilled, deionized water. The above solution is the minimum copper sulphate concentration required to catalyze the reduction reaction. If an excess is added, it will very slowly precipitate and re-dissolve cyclically; this causes loss of stability as shown by variation of the in-run standards.

4.18. Color Reagent

To approximately 500 ml of distilled, deionized water, add 50 ml of concentrated hydrochloric acid. Dissolve 7.50 g of sulphanilamide and 0.375 g of N(1-naphthyl) ethylenediamine dihydrochloride in the acid solution and dilute to 1.0 liter. Filter if necessary.

THIS PROCEDURE MUST BE DONE IN A FUME HOOD WITH ADEQUATE SAFETY PRECAUTIONS TAKEN TO PROTECT EYES AND OTHER EXPOSED AREAS AGAINST ACID SPILLS.

AS ALL NAPHTHYL TYPE COMPOUNDS ARE POTENTIAL CARCINOGENS, CARE SHOULD BE TAKEN IN HANDLING AND WEIGHING N(1-NAPHTHYL) ETHYLENEDIAMINE DIHYDROCHLORIDE.

4.19. Concentrated Stock Standard Nitrate Solution

Dissolve 12.99 g of anhydrous reagent grade potassium nitrate, KNO_3 , in distilled, deionized water and dilute to 1 liter in a volumetric flask. Mix well and store in a tightly stoppered container under refrigeration. Concentration of NO_3 : 1800 mg/l as N.

4.20. Concentrated Stock Standard Nitrite Solution

Dissolve 1.971 g reagent grade sodium nitrite, NaNO_2 , in distilled, deionized water and dilute to 2 liters in a volumetric flask. Mix well and store in a tightly stoppered container under refrigeration. Concentration of NO_2 : 200 mg/l as N.

4.21. Intermediate Stock Standard Nitrate Plus Nitrite Solution

Transfer 20.0 ml aliquots of both the nitrate and nitrite concentrated stock standards to 1 liter volumetric flask, and dilute to volume with distilled, deionized water. Concentration of NO_3 plus NO_2 : 40.0 mg/l as N. This standard may be used for one working week.

NOTE: In actual practice one intermediate stock solution is prepared for the calibration of all four nutrient channels; thus this standard also contains ammonia, 16.0 mg/l as N, and orthophosphate, 4.00 mg/l as P.

4.22. Working Calibration Standards

Three working standards are prepared daily from the intermediate stock standard solution:

Low (L): dilute 5.00 ml of intermediate stock to 1 liter with distilled, deionized water. Concentrations of nitrate and nitrite are 0.180 mg/l as N and 0.020 mg/l as N respectively.

- Medium (M): dilute 20.0 ml of intermediate stock to 1 liter with distilled, deionized water. Concentrations of nitrate and nitrite are 0.720 mg/l as N and 0.080 mg/l as N respectively.
- High (H): dilute 100.0 ml of intermediate stock to 1 liter with distilled, deionized water. Concentrations of nitrate and nitrite are 3.60 mg/l as N and 0.40 mg/l as N respectively.

4.23. Control Solutions for Reduction Step

- 4.23.1. Prepare stock nitrate control solution by dissolving 7.2187 g KNO_3 in 1 liter of distilled, deionized water. Concentration of nitrate: 1000 mg/l as N.
- 4.23.2. Prepare intermediate nitrate control solution by diluting 10.0 ml of stock nitrate control solution to 1 liter with distilled, deionized water. Concentration of nitrate: 10.0 mg/l as N.
- 4.23.3. Prepare working nitrate control solution by diluting 50.0 ml of intermediate nitrate control solution to 1 liter with distilled, deionized water. Concentration of nitrate: 0.500 mg/l as N.
- 4.23.4. Prepare stock nitrite control solution by dissolving 4.9262 g NaNO_2 in 1 liter of distilled, deionized water. Concentration of nitrite: 1000 mg/l as N.
- 4.23.5. Prepare intermediate nitrite control solution by diluting 10.0 ml of stock nitrite control solution to 1 liter with distilled, deionized water. Concentration of nitrite: 10.0 mg/l as N.
- 4.23.6. Prepare working nitrite control solution by diluting 50.0 ml of intermediate control solution to 1 liter with distilled, deionized water. Concentration of nitrite: 0.500 mg/l as N.

4.24. Control Solutions for Checking Divalent Cation Removal

- 4.24.1. Prepare stock calcium ion control solution by dissolving 3.7 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 1 liter of distilled, deionized water.
- 4.24.2. Prepare stock magnesium ion control solution by dissolving 10 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 liter of distilled, deionized water.
- 4.24.3. Prepare working cation control solution by diluting 50.0 ml of intermediate nitrate control solution, 50.0 ml of stock calcium ion control solution, and 50.0 ml of stock magnesium ion control solution to 1 liter with distilled, deionized water. Concentrations of major ions: 0.500 mg/l nitrate as N, 150 mg/l calcium ion as Ca and 50 mg/l magnesium ion as Mg.

4.25. Quality Control Solutions

For each analytical range, two quality control (QC) solutions are required to confirm calibration. One long term blank (the water used to prepare the working QC solutions) serves both ranges. The concentrations of the QC solutions are chosen such that they cover the normal concentration range of samples being routinely analyzed. Sufficient volumes are prepared to last a minimum of 20 days of analyses; whenever new QC solutions are required, they are prepared in advance so that they may be monitored for at least three days prior to adopting them.

5. Procedure

- 5.1. Collect the samples and group them according to the bench sheet.
- 5.2. Prepare the filtration apparatus as shown in Figure 1.
- 5.3. Using only forceps, place the appropriate glass fibre filter in the funnel.
- 5.4. Fit a clean 1.8 x 15.0 cm culture tube onto the adaptor, being careful not to touch the rim or inside glass surface of the tube or the rubber adaptor.
- 5.5. Shake the sample vigorously and promptly vacuum filter two 15 ml portions, discarding the filtrate each time.
- 5.6. Collect a third portion of the filtrate for analysis. Remove the contaminated filter paper with the forceps.
- 5.7. Place the culture tube, containing the filtered sample, into a test tube rack in such a position that it may easily be correlated with the sample number on the bench sheet. Write the appropriate sample number on the first and last sample tube in each row.

5.8. "Amberlite" IR-120 Ion Exchange Column

Loosely pack a Pyrex glass tube, 0.3 cm I.D. x 8 cm length with Amberlite IR-120 ion exchange resin which is kept stored in a solution of 10% (V/V) hydrochloric acid. Ends of the tube are loosely plugged with glass wool. To pack the tube, draw resin from the acid solution through the tube by vacuum attached to one (plugged) end. The column must be regenerated daily by drawing 10% (V/V) hydrochloric acid through for about 5 min. If two columns are alternated daily, they need to be repacked after 1 week of normal use.

- 5.9. Set the AutoAnalyzer into operation using cleaning and checking procedure appropriate to the manifold illustrated in Figure 2. For the ion exchange column, care must be taken to ensure that no air bubbles are present or the peaks will be noisy. By using a small column, an excellent wash can be obtained with an analysis rate of 24 per hour.
- 5.10. When loading the samples into the AutoAnalyzer sampler module, ensure that sample order conforms to the bench sheet.
- 5.11. Each run of samples includes all of the following units:

Set of calibration standards: H, M, L
Distilled water blank (day's supply): B1
Quality Control samples: QC-A, QC-B, QC-C, QC-D
Long term blank: LTBI
Reduction control solutions: 0.5 mg/l NO_3 as N, 0.5 mg/l NO_2 as N
Cation control solution: 0.5 mg/l NO_3 as N plus 150 mg/l calcium as Ca and 50 mg/l magnesium as Mg

The basic sample loading sequence is: 10 samples, B1, 10 samples, L, M, H, B1.

- 5.12. Calibrate the AutoAnalyzer system using calibration standards. Record the standard calibration setting, and check to ensure that it has not changed unduly.

- 5.13. Analyze the working control solutions for divalent cation removal and for the reduction step. Repeat these analyses at the end of the day's run. The peaks for the nitrate (0.500 mg/l as N) and nitrite (0.500 mg/l as N) must not differ by more than 4%. If the difference exceeds 4%, the run must be stopped. When the problem has been solved, the system must be recalibrated.
- 5.14. The peaks for the nitrate (0.500 mg/l as N) and cation control solution (0.500 mg/l nitrate as N, 150 mg/l calcium as Ca and 50 mg/l magnesium as Mg) must not differ by more than 2% and the peak for the latter must not be deformed. If these conditions are not met, a fresh ion exchange column must be introduced. When the defect has been corrected, the system must be recalibrated.
- 5.15. Confirm the calibration by analyzing the quality control solutions. Record these values. For each analytical range, calculate totals and differences, e.g., QC-C plus QC-D and QC-C minus QC-D. Check to ensure that the calculated values conform to limits based on past data.
- 5.16. Monitor calibration standards (in-run sensitivity checks) throughout the run to determine if within-run corrections are required.
- 5.17. Read sample peak heights, and convert to concentration values.
- 5.18. **Troubleshooting**
 - 5.18.1. For this methodology, nitrate is reduced quantitatively to nitrite but nitrite is not reduced. When the system is optimized, the recovery of nitrite is not changed by replacing the flow of hydrazine with an equal flow of distilled, deionized water. As the channel is calibrated with mixed standards containing both nitrate and nitrite, the system must be optimized even though the nitrite concentrations of samples are very low.
 - 5.18.2. Copper precipitates on the mixing coil during the course of the day's run; during the early stages, precipitation tends to increase the sensitivity of the system and, if excessive, it will cause noisy peaks. To minimize these effects the system is cleaned daily by pumping 10% HCl (5 min), then distilled water (20 min.) and finally reagents (20 min.).
 - 5.18.3. The temperature of the heating bath must be 38°C.
 - 5.18.4. The pH of the stream leaving the constant temperature bath must be 12.2 ± 0.1 units while the pH of the waste stream from the colorimeter should be 0.9 ± 0.1 units.
 - 5.18.5. The hydrazine and copper sulphate concentrations are critical; check pump tubes frequently for wear.
 - 5.18.6. The working hydrazine reagent must be prepared daily. The position of the hydrazine in the manifold (just before heating bath) must not be changed without a full re-evaluation of the system.

6. Calculation and Reporting

If required, multiply the reading by the dilution factor

$$\frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result in the answer space opposite each sample number of the bench sheet. Report the results to three significant figures if feasible.

7. Precision and Accuracy

Precision - duplicate analyses of routine samples

<u>Range</u>	<u>Sample Concentration Range</u> (mg/l as N)	<u>Standard Deviation</u> (mg/l as N)
Low	< 0.20	0.0060
	0.20 - 0.50	0.0071
	0.50 - 1.00	0.0089
High	1.00 - 2.50	0.0043
	2.50 - 5.00	0.0224

Accuracy-recovery of quality control (QC) solutions

<u>Range</u>	<u>QC Concentration</u> (mg/l as N)	<u>Average Recovery</u> (mg/l as N)	<u>Standard Deviation</u> (mg/l as N)
Low	0.250	0.252	0.0073
	0.750	0.754	0.0114
High	1.250	1.276	0.0275
	3.750	3.749	0.0622

8. Bibliography

- 8.1. Kamphake, L. J., Hannah, S. A., and Cohen, J. M., Automated Analysis for Nitrate by Hydrazine Reduction, Water Research 1, 205, 1967.
- 8.2. Methods for Chemical Analyses of Water and Wastes, E.P.A., Storet No. 00630, Page 185, 1971.
- 8.3. Jacobs, M. B., and Hochheiser, S., Continuous Sampling and Ultramicro Determination of Nitrogen Dioxide in Air, Anal. Chem. 30, 426, 1958.
- 8.4. Crowther, J., McBride, J., and Wright, B. Low Level Hydrazine Procedure for Nitrate Plus Nitrite, Internal Ministry of the Environment Report. March 7, 1978.

Hydrazine Reduction - Azo Dye Method A

Variation #1

SUMMARY

Matrix.	Variation #1 is used routinely for domestic water, sewages, leachates and industrial waste.
Substance determined.	Nitrate (NO_3^-) and Nitrite (NO_2^-) ions.
Interpretation of results.	Results are reported in mg/l as N. The nitrite fraction is generally less than five percent of the nitrate concentration. For domestic water supplies, the result must be less than 10 mg/l as N in order to meet the nitrate criteria.
Principle of method.	Nitrate plus nitrite nitrogen is determined as one of two automated tests which are performed simultaneously on the same aliquot of supernatant from a sample. The nitrate content of the sample is reduced to nitrite by heating an aliquot with hydrazine in alkaline media; this reaction is catalyzed by the addition of cupric ion. Subsequently, an azo dye is formed in acid media by diazotizing sulphanilamide with nitrite and coupling the product with N(1-naphthyl) ethylenediamine dihydrochloride. The absorbance of the light red azo dye is measured at 520 nm and the concentration of nitrate plus nitrite is determined by comparison with a similarly treated series of mixed standards.
Time required for analysis.	Approximately 24 analyses can be performed in an hour and over 200 per day.
Range of application.	Dual ranges for undiluted sample: 0.1 - 20.0 mg/l as N and 20.0 - 50.0 mg/l as N. Higher levels are determined on diluted samples.
Standard deviation.	Based on duplicate analyses, the average standard deviations for the low and high ranges are 0.12 and 0.38 mg/l as N respectively.
Accuracy.	The average recovery of three standards is 99.9% with relative standard deviations ranging from 0.9 to 1.5%.
Detection criteria.	0.11 mg/l as N.
Interferences and shortcomings.	Excessive concentrations of oxidizing or reducing agents will interfere with the reduction step. Interferences from divalent cations are eliminated by use of an ion exchange column.
Minimum volume of sample.	75 ml.

**Preservation and
sample container.**

Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

At room temperature bacteria can convert organic nitrogen to ammonia, and ammonia to nitrite. These reactions are accelerated for domestic wastes due to the abundance of bacteria.

**Safety
considerations.**

Normal safety precautions for handling strong bases and acids are required. As all naphthyl type compounds are considered potential carcinogens until proven otherwise, care should be taken in handling the powder, N(1-naphthyl) ethylenediamine dihydrochloride.

Hydrazine Reduction - Azo Dye Method A**Variation #1****1. Introduction**

The nitrate plus nitrite contents of domestic water supplies, sewages, leachates, and industrial wastes are determined by a less sensitive variation of Method A. The changes entail inclusion of a dilution loop and analyses of sample supernatant rather than filtrate.

2. Interferences and Shortcomings

Excessive concentrations of oxidizing or reducing agents will interfere with the reduction step. Interferences from divalent cations are eliminated by use of an ion exchange column.

3. Apparatus (Changes)

- 3.1. Manual filtration unit is eliminated.
- 3.2. Ion exchange column is larger, i.e., 0.3 cm diameter x 15 cm length.
- 3.3. Pump tubing and glassware as indicated in Figure 3.
- 3.4. AAI colorimeter flow cell is 1.5 cm.

4. Reagents (Changes)

- 4.1. Calibration standards contain all four dissolved nutrients, but are prepared by diluting stock solutions of the individual components. For the working standards, the nitrate plus nitrite concentrations are 40.0 mg/l as N and 16.0 mg/l as N.
- 4.2. The two control solutions for the reduction step are (1) nitrate: 10.0 mg/l as N and (2) nitrite: 10.0 mg/l as N.
- 4.3. The control solution for checking divalent cation removal contains nitrate: 10.0 mg/l as N, calcium ion: 150 mg/l as Ca, and magnesium ion: 50 mg/l as Mg.
- 4.4. Working quality control solutions are prepared from concentrated stock solutions of the individual nutrients. The working QC solutions are used for all four soluble nutrient channels. Nitrite is omitted from QC-A as it is not required for the nitrite channel (single range) and high nitrite concentrations are an interference for filtered reactive phosphorus.

5. Procedure

Samples are not filtered, but the probe of the sampler is set so that only the supernatant is drawn into the manifold. Analyses are completed in accordance with the standard procedure for AutoAnalyzers (see Method A).

6. Calculation and Reporting

If required, multiply the reading by the dilution factor

$$\frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result in the answer space opposite each sample number of the bench sheet. Report the results to three significant figures if feasible.

7. Precision and Accuracy

Precision - duplicate analyses of routine samples

<u>Range</u>	<u>Sample Concentration Range</u> (mg/l as N)	<u>Standard Deviation</u> (mg/l as N)
Low	< 4.00	0.066
	4.00 - 10.0	0.120
	10.0 - 20.0	0.272
High	20.0 - 25.0	-
	25.0 - 50.0	0.382

Accuracy-recovery of quality control (QC) solutions

<u>Range</u>	<u>QC Concentration</u> (mg/l as N)	<u>Average Recovery</u> (mg/l as N)	<u>Standard Deviation</u> (mg/l as N)
Low	4.20	4.08	0.065
	15.40	15.57	0.156
High	15.40	15.56	0.168
	35.00	35.38	0.318

8. Bibliography

- 8.1. Kamphake, L. J., Hannah, S. A., and Cohen, J. M., Automated Analysis for Nitrate by Hydrazine Reduction, Water Research 1, 205, 1967.
- 8.2. Methods for Chemical Analyses of Water and Wastes, E.P.A., Storet No. 00630, Page 185, 1971.
- 8.3. Jacobs, M. B., and Hochheiser, S., Continuous Sampling and Ultramicro Determination of Nitrogen Dioxide in Air, Anal. Chem. 30, 426, 1958.
- 8.4. Crowther, J., McBride, J., and Wright, B. Low Level Hydrazine Procedure for Nitrate Plus Nitrite, Internal Ministry of the Environment Report. March 7, 1978.

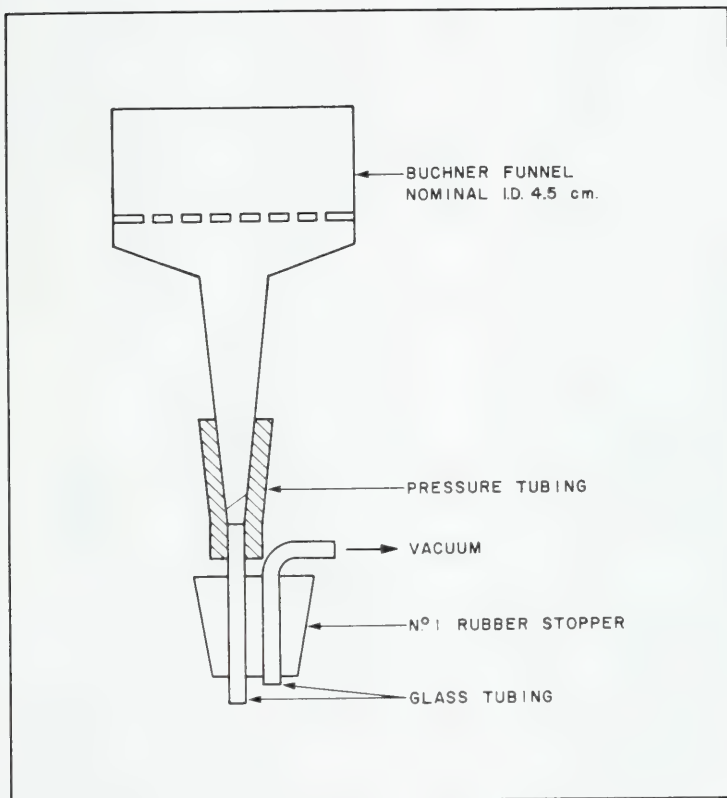


FIGURE 1 - FILTRATION APPARATUS FOR NITRATE NITROGEN PLUS NITRITE NITROGEN, METHOD A.

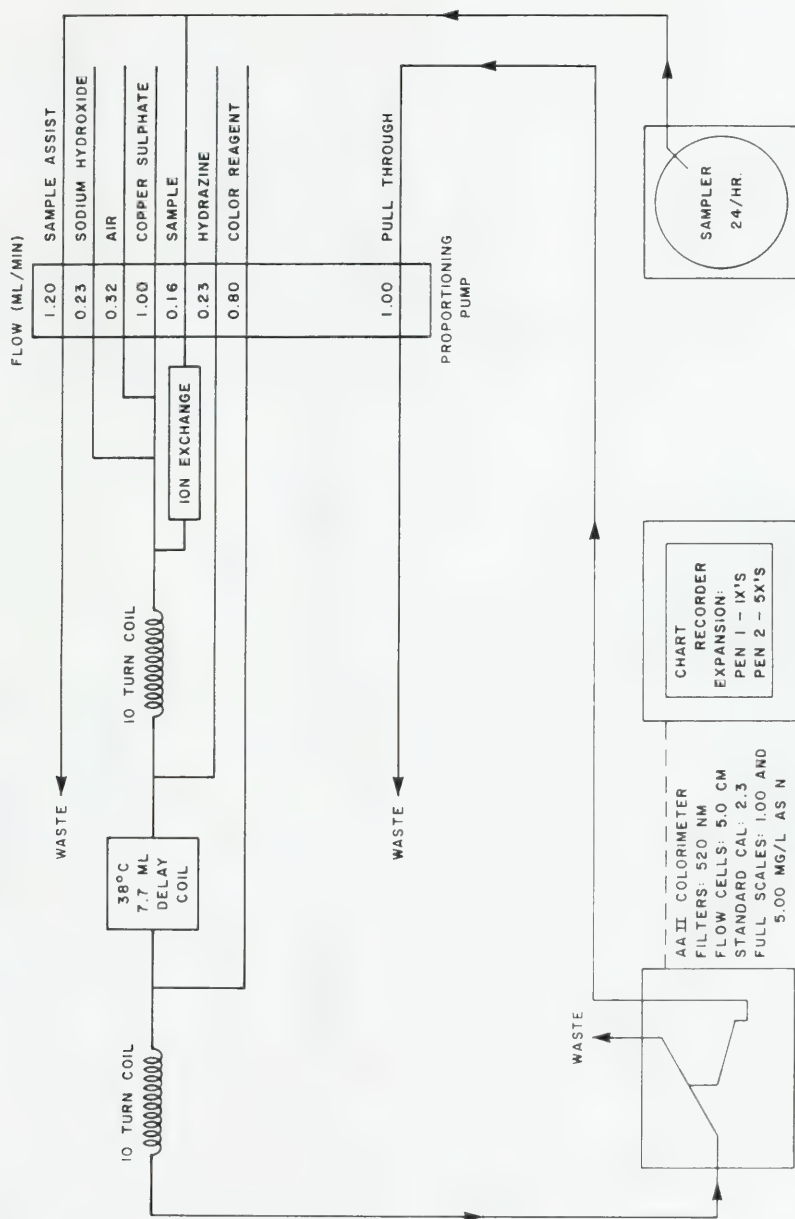


FIGURE 2 - AUTOMATED SYSTEM FOR FILTERED NITRATE PLUS NITRITE NITROGEN, METHOD A.

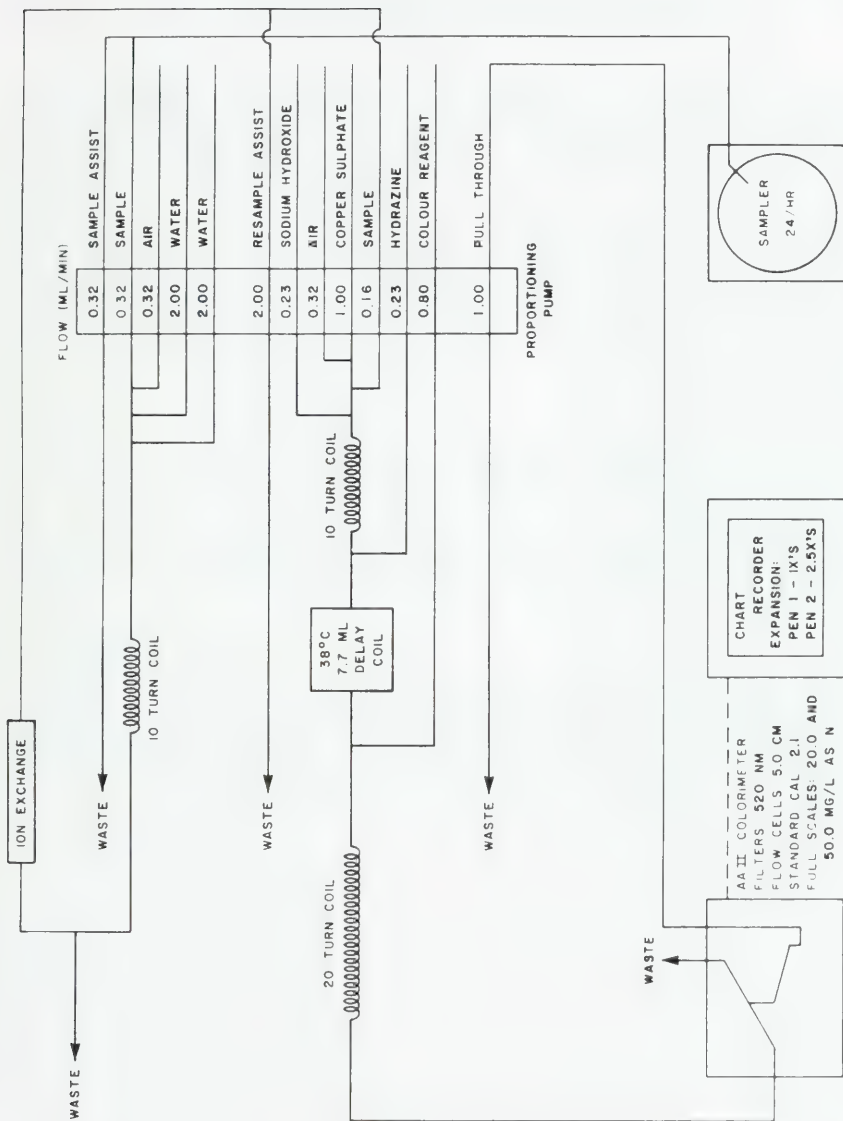


FIGURE 3 - AUTOMATED SYSTEM FOR NITRATE PLUS NITRITE NITROGEN, VARIATION #1 OF METHOD A.

THE DETERMINATION OF NITRITE NITROGEN

Nitrite is present in natural waters as an intermediary in the oxidation of ammonia by autotrophic nitrifying bacteria or in the anaerobic reduction of nitrate. In either case, nitrite concentrations rarely exceed 0.1 mg/l as N in natural systems. Higher concentrations usually reflect the presence of an industrial effluent which is creating rapid chemical changes in the natural balance of nutrients.

Nitrite results may be used in interpreting the effectiveness of the waste stabilization processes, particularly at sewage treatment facilities. However, the dynamic nature of the nitrogenous interconversions in many samples must be considered, and full allowance made for the extreme perishability of this parameter. Nitrite exists in water only in the dissolved, ionic form and a nitrite determination is performed on a filtered portion of the sample or on the sample supernatant.

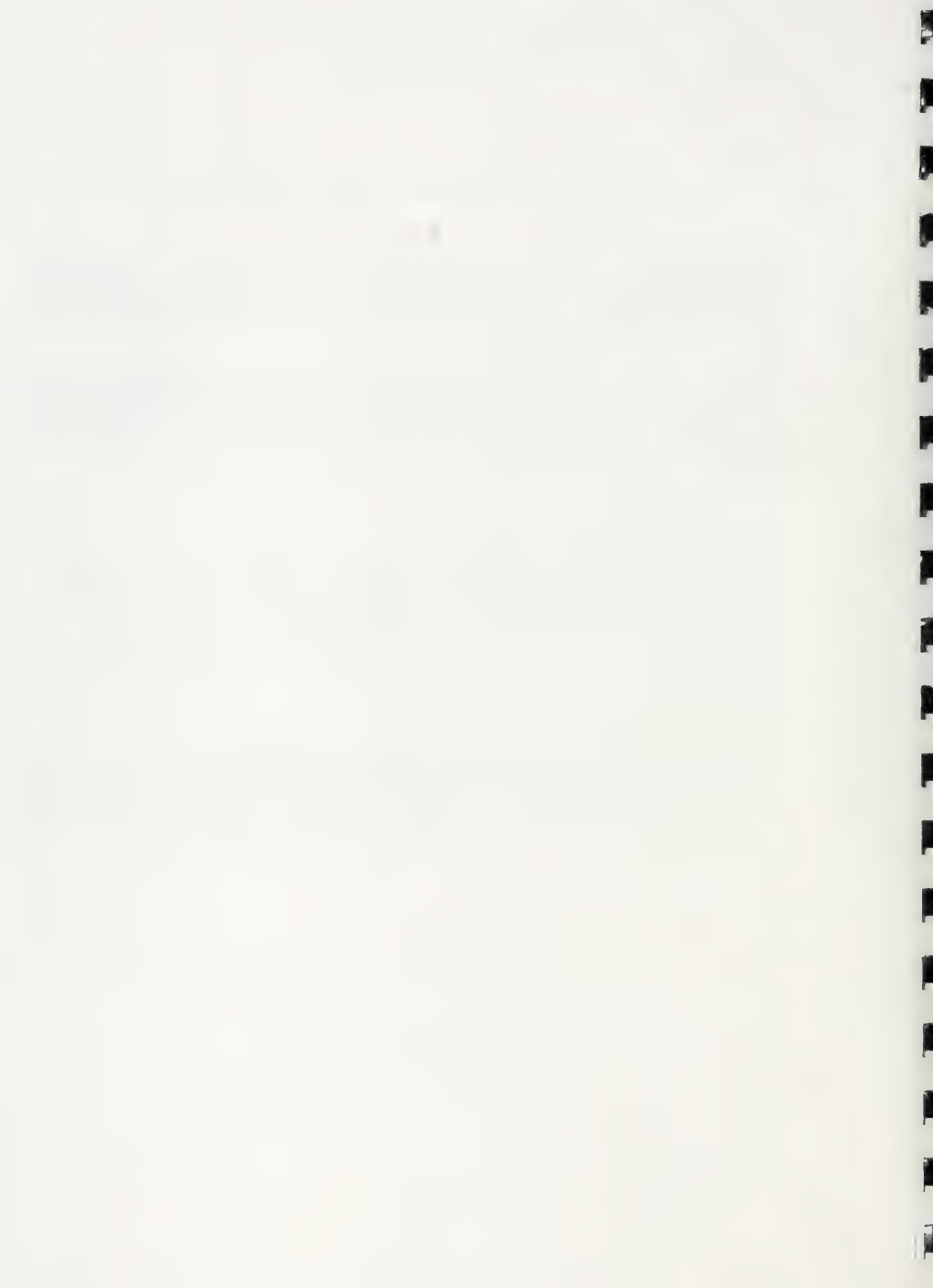
Sample Handling and Preservation

Glass or plastic bottles are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

At room temperature bacteria can convert organic nitrogen to ammonia, and ammonia to nitrite. These reactions are accelerated for domestic wastes due to the abundance of bacteria.

Selection of Method

Method A, used for clean rivers and lakes samples, is a colorimetric method based on the formation of azo dye. The colorimetry portion of the analysis is performed automatically using an AutoAnalyzer system. The range of application is limited only by the dilution required to obtain a satisfactory colorimetric response. For domestic waters, sewages and industrial wastes, a less sensitive variation of Method A is used (Variation #1).



NITRITE NITROGEN

Azo Dye Method A

SUMMARY

Matrix.	Method A is used routinely for clean rivers and lakes.
Substance determined.	Nitrite ion, NO_2^- .
Interpretation of results.	In nature, a nitrite concentration greater than 0.1 mg/l as N is rare because nitrite ion is an intermediate product of the biological decomposition of proteinaceous material. Even in wastewater, nitrite tends to be less than 1 mg/l as N. Conversion to nitrate or lower valency states of nitrogen is to be expected.
Principle of method.	Nitrite is determined as one of four automated nutrient tests performed simultaneously on the same aliquot of filtered sample. Nitrite forms a diazotization product with sulphanilamide which is then coupled with N(1-naphthyl) ethylenediamine dihydrochloride at $\text{pH } 1 \pm 0.1$. A light red color is produced. The absorbance of the solution is measured at 520 nm and the concentration of nitrite is determined by comparison with a known set of standards.
Time required for analysis.	Approximately 24 analyses can be performed in an hour. Operation of the AutoAnalyzer equipment can be maintained for as long as 18 hours a day.
Range of application.	0.0018 - 0.100 mg/l as N on undiluted samples. Concentrations exceeding 0.100 mg/l as N are determined by dilution of the filtered sample.
Standard deviation.	The average standard deviation for duplicate analyses is 0.0006 mg/l as N.
Accuracy.	The average recovery of quality control solutions is 102% with a relative standard deviation of 2.1%.
Detection criteria.	0.0018 mg/l NO_2^- as N.
Interferences and shortcomings.	Nitrogen trichloride and free available chlorine are positive interferences.

**Minimum volume
of sample.**

75 ml.

**Preservation and
sample container.**

Glass, polyethylene, or polystyrene bottles are acceptable. Refrigeration and rapid shipment of samples is essential. Acid preservation must be avoided.

**Safety
considerations.**

Naphthyl compounds are suspected to be carcinogenic. Thus N (1-naphthyl) ethylenediamine dihydrochloride should be handled with care.

NITRITE NITROGEN

Azo Dye Method A

1. Introduction

This procedure is designed for relatively clean rivers and lakes.

A filtered portion of sample is presented to the AutoAnalyzer where a proportioned aliquot is withdrawn into the nitrite channel. The sample stream is mixed with an air segmented color reagent which contains N(1-naphthyl) ethylenediamine dihydrochloride and sulphanilamide. The nitrite ions in the sample react with sulphanilamide to form a diazo compound, which in turn couples with the naphthyl compound to produce a light red chromophore in proportion to the concentration of nitrite present in the sample. The absorbance of the solution is measured with a colorimeter at 520 nm with a 5 cm flow cell and recorded as a peak on a chart recorder. The nitrite result in mg/l as N is read from the chart recorder by comparison with peaks produced by similarly treated standards.

2. Interferences and Shortcomings

Nitrogen trichloride and free available chlorine can give false positive results. Their absence should be ensured by dechlorination. As in other colorimetric tests, colored substances should be absent.

3. Apparatus

- 3.1. Filtration apparatus constructed as shown in Figure 1.
- 3.2. Glass fibre filters, Reeve Angel 934AH, 4.25 cm diameter.
- 3.3. Automated sampler.
- 3.4. Proportioning pump.
- 3.5. Colorimeter, equipped with 520 nm filters and 5.0 cm flow cells.
- 3.6. Voltage regulator.
- 3.7. Chart recorder.
- 3.8. Pump tubing and associated glassware as indicated in Figure 2.
- 3.9. Culture tubes, 1.8 x 15.0 cm.
- 3.10. Culture tube racks of 40 tube capacity.
- 3.11. Dilution tubes, 50 ml capacity.

4. Reagents

- 4.1. Sodium nitrite, NaNO_2 , reagent grade crystals.
- 4.2. N(1-Naphthyl) ethylenediamine dihydrochloride, $\text{C}_{10}\text{H}_7\text{HNCH}_2\text{CH}_2\text{NH}_2 \cdot 2\text{HCl}$, reagent grade powder.

NOTE: HANDLE THE POWDERED REAGENT CAREFULLY - IT IS A POTENTIAL CARCINOGEN.

- 4.3. Sulphanilamide, $\text{NH}_2 \cdot \text{C}_6\text{H}_4 \cdot \text{SO}_2\text{NH}_2$, reagent grade powder.
- 4.4. Hydrochloric acid, HCl , concentrated reagent grade.

4.5. Color Reagent

To approximately 500 ml of distilled, deionized water, add 30 ml of concentrated hydrochloric acid. Dissolve 7.5 g of sulphanilamide and 0.375 g of N(1-naphthyl) ethylenediamine dihydrochloride in the acid solution and dilute to 1.0 liter. Filter if necessary.

NOTE: This procedure must be done in a fume hood with adequate safety precautions taken to protect eyes and other exposed areas against acid spills.

4.6. Concentrated Stock Standard Nitrite Solution

Dissolve 0.986 g of sodium nitrite in distilled, deionized water and dilute to 1 liter in a volumetric flask. Concentration of nitrite is 200 mg/l as N.

4.7. Intermediate Stock Standard Nitrite Solution

Dilute a 20.0 ml aliquot of the concentrated stock standard nitrite solution to 1 liter in a volumetric flask. Concentration of nitrite is 4.00 mg/l as N. In actual practice, this stock also contains ammonia: 16 mg/l as N, nitrate: 36 mg/l as N, and orthophosphate: 4 mg/l as P. This standard is good for one week.

4.8. Working Calibration Standards

Daily calibration standards are prepared by diluting the intermediate stock standard:

Low (L): 5.00 ml of the intermediate stock standard are diluted to 1 liter in a volumetric flask. Concentration of nitrite is 0.020 mg/l as N.

Medium (M): 20.0 ml of the intermediate stock standard are diluted to 1 liter in a volumetric flask. Concentration of nitrite is 0.080 mg/l as N.

4.9. Quality Control Solutions

Two quality control (QC) solutions are analyzed daily; a long term blank consisting of the water used to prepare the working QC solutions is also analyzed daily. Sufficient volumes are prepared to last a minimum of 20 days of analyses; whenever new QC solutions are required, they are prepared in advance so that they may be monitored for at least three days prior to adopting them.

5. Procedure

- 5.1. Collect the samples and group them according to the bench sheet.

- 5.2. Prepare the filtration apparatus as shown in Figure 1.
- 5.3. Using only forceps, place the appropriate glass fibre filter in the funnel.
- 5.4. Fit a clean 1.8 x 15.0 cm culture tube onto the adaptor, being careful not to touch the rim or inside glass surface of the tube or the rubber adaptor.
- 5.5. Shake the sample vigorously and promptly vacuum filter two 15 ml portions, discarding the filtrate each time.
- 5.6. Collect a third portion of the filtrate for analysis. Remove the contaminated filter paper with the forceps.
- 5.7. Place the culture tube containing the filtered sample into a test tube rack in such a position that it may easily be correlated with the sample number on the bench sheet. Write the appropriate sample number on the first and last sample tube in each row.
- 5.8. Set the AutoAnalyzer into operation using cleaning, set-up and checking procedures appropriate to the manifold illustrated in Figure 2.
- 5.9. When loading the samples into the AutoAnalyzer sampler module, ensure that sample order conforms to the bench sheet.
- 5.10. Each run of samples will include all of the following units:
 - Set of calibration standards: M, L
 - Distilled water blank (day's supply): B1
 - Quality Control samples: QC-A, QC-B
 - Long term blank: LTBI

The sample loading sequence is: 10 samples, B1, 10 samples, L, M, B1.
- 5.11. Calibrate the AutoAnalyzer system using calibration standards. Record the standard calibration setting, and check to ensure that it has not changed unduly.
- 5.12. Confirm the calibration by analyzing the quality control solutions. Record these values. For each analytical range, calculate totals and differences, e.g., QC-A plus QC-B and QC-A minus QC-B. Check to ensure that the calculated values conform to limits based on past data.
- 5.13. Monitor calibration standards (in-run sensitivity checks) throughout the run to determine if within-run corrections are required.
- 5.14. Read sample peak heights, and convert to concentration values.

6. Calculation and Reporting

If required, multiply the reading in mg/l as N by the dilution factor

$$\frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result in the answer space opposite each sample number of the bench sheet. Report the results to three significant figures if feasible.

7. Precision and Accuracy

Precision-duplicate analyses of routine samples

Sample Concentrations (mg/l as N)	Standard Deviation (mg/l as N)
<0.020	0.00109
0.020 - 0.050	0.00084
0.050 - 0.100	-

Accuracy-recovery of quality control (QC) solutions

QC Concentration (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
0.075	0.0745	0.00128
0.025	0.0262	0.00064

8. Bibliography

- 8.1. Kamphake, L.J., Hannah, S.A., and Cohen, J.M., Automated Analysis for Nitrate by Hydrazine Reduction, Water Research 1, 205, 1967.
- 8.2. Methods of Chemical Analyses of Water and Wastes, E.P.A., Storet No. 00630, Page 185, 1971.
- 8.3. Jacobs, M.B., and Hochheiser, S., Continuous Sampling and Ultramicro Determination of Nitrogen Dioxide in Air, Anal. Chem. 30, 426, 1958.
- 8.4. Standard Methods for the Examination of Water and Wastewater, APHA, Washington, 13th edition, 1972, 240.
- 8.5. "Evaluation of a Dual-Channel Automated Procedure for Nitrate and Nitrate Analysis Based Upon the Reduction of Nitrate to Nitrite And/Or Colourimetric Analysis for Nitrite" Internal Ministry of the Environment Report by J. Crowther and B. Wright, November, 1975.

NITRITE NITROGEN

Azo Dye Method A

Method A Variation #1

SUMMARY

Matrix.	Method A Variation #1 is used routinely for domestic water, sewage and industrial wastes.
Substance determined.	Nitrite ion, NO_2^- .
Interpretation of results.	A nitrite concentration greater than 1.0 mg/l as N is rare since nitrite ion is an unstable intermediate product of the biological decomposition of proteinaceous material. Rapid conversion to nitrate is to be expected.
Principle of method.	Nitrite nitrogen is determined as one of two automated tests which are performed simultaneously on the sample aliquot of supernatant from a settled sample. Nitrite forms a diazotization product with sulphanilamide which is then coupled with N(1-naphthyl) ethylenediamine dihydrochloride at $\text{pH } 1 \pm 0.1$. The absorbance of the light red solution is measured at 520 nm and the concentration of nitrite nitrogen is determined by comparison with a similarly treated series of standards.
Time required for analysis.	Approximately 24 analyses can be performed in an hour.
Range of application.	0.009 - 2.00 mg/l as N. Higher concentrations are determined by dilution.
Standard deviation.	The average standard deviation for duplicate analyses is 0.0124 mg/l as N.
Accuracy.	Recovery of quality control solutions is 100% with a relative standard deviation of one per cent.
Detection criteria.	0.015 mg/l as N.

Interferences and shortcomings.

Free chlorine and nitrogen trichloride.

Minimum volume of sample.

75 ml.

Preservation and sample container.

Glass, polyethylene, or polystyrene bottles are acceptable. Refrigeration and rapid shipment of samples is essential.

Safety considerations.

Normal safety precautions for handling strong bases and acids are required. As all naphthyl type compounds are considered potential carcinogens until proven otherwise, care should be taken in handling the powder, N (1-naphthyl) ethylenediamine dihydrochloride.

NITRITE NITROGEN

Azo Dye Method A

Method A Variation #1

1. Introduction

For domestic water supplies, sewages and industrial wastes, a less sensitive variation of Method A is used. This entails analyzing the supernatant of a settled sample instead of the filtrate, and includes a 13.5x's dilution loop in the manifold.

2. Interferences and Shortcomings

Nitrogen trichloride and free available chlorine can give false positive results. Their absence should be ensured by dechlorination. As in other colorimetric tests, colored substances should be absent.

3. Apparatus (Changes)

- 3.1. Manual filtration unit is eliminated.
- 3.2. Dilution loop is added to manifold (Figure 3).

4. Reagents (Changes)

- 4.1. Calibration standards contain all four dissolved nutrients, but are prepared by diluting stock solutions of the individual components. For the working standards, the nitrite nitrogen concentration is 1.60 mg/l as N.
- 4.2. Quality control solutions are prepared from another set of stocks. Nitrite nitrogen concentrations are appropriate for the range of application. A long term blank of the water used to prepare the working quality control solutions is analyzed daily.

5. Procedure

- 5.1. Samples are not filtered, but the probe of the sampler is set so that only the supernatant is drawn into the manifold.
- 5.2. Analyses are completed in accordance with the standard procedure for AutoAnalyzers (See Method A).

6. Calculation and Reporting

If required, multiply the reading by the dilution factor

$$\frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result in the answer space opposite each sample number of the bench sheet. Report the results to three significant figures if feasible.

7. Precision and Accuracy

Precision - duplicate analyses of routine samples

Sample Concentration Range (mg/l as N)	Standard Deviation (mg/l as N)
< 0.40	0.0090
0.40 - 1.00	0.0204
1.00 - 2.00	0.0244

Accuracy-recovery of quality control (QC) solutions

QC Concentration (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
0.70	0.688	0.0237
1.40	1.399	0.0259

8. Bibliography

- 8.1. Kamphake, L.J., Hannah, S.A., and Cohen, J.M., Automated Analysis for Nitrate by Hydrazine Reduction, Water Research 1, 205, 1967.
- 8.2. Methods of Chemical Analyses of Water and Wastes, E.P.A., Storet No. 00630, Page 185, 1971.
- 8.3. Jacobs, M.B., and Hochheiser, S., Continuous Sampling and Ultramicro Determination of Nitrogen Dioxide in Air, Anal. Chem. 30, 426, 1958.
- 8.4. Standard Methods for the Examination of Water and Wastewater, APHA, Washington, 13th edition, 1972, 240.
- 8.5. "Evaluation of a Dual-Channel Automated Procedure for Nitrate and Nitrate Analysis Based Upon the Reduction of Nitrate to Nitrite And/OR Colourimetric Analysis for Nitrite" Internal Ministry of the Environment Report by J. Crowther and B. Wright, November, 1975.

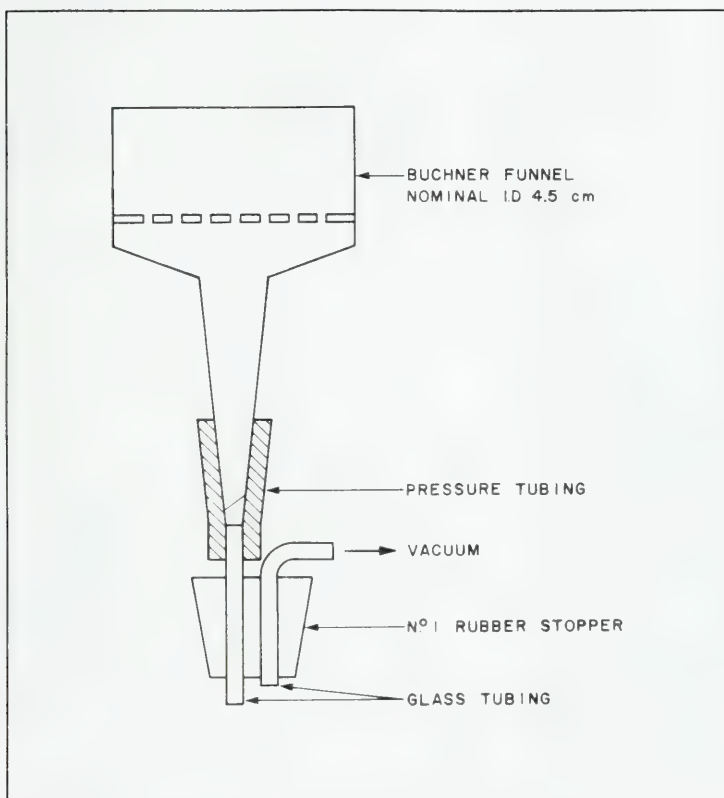


FIGURE 1 - FILTRATION APPARATUS FOR NITRITE
NITROGEN, METHOD A.

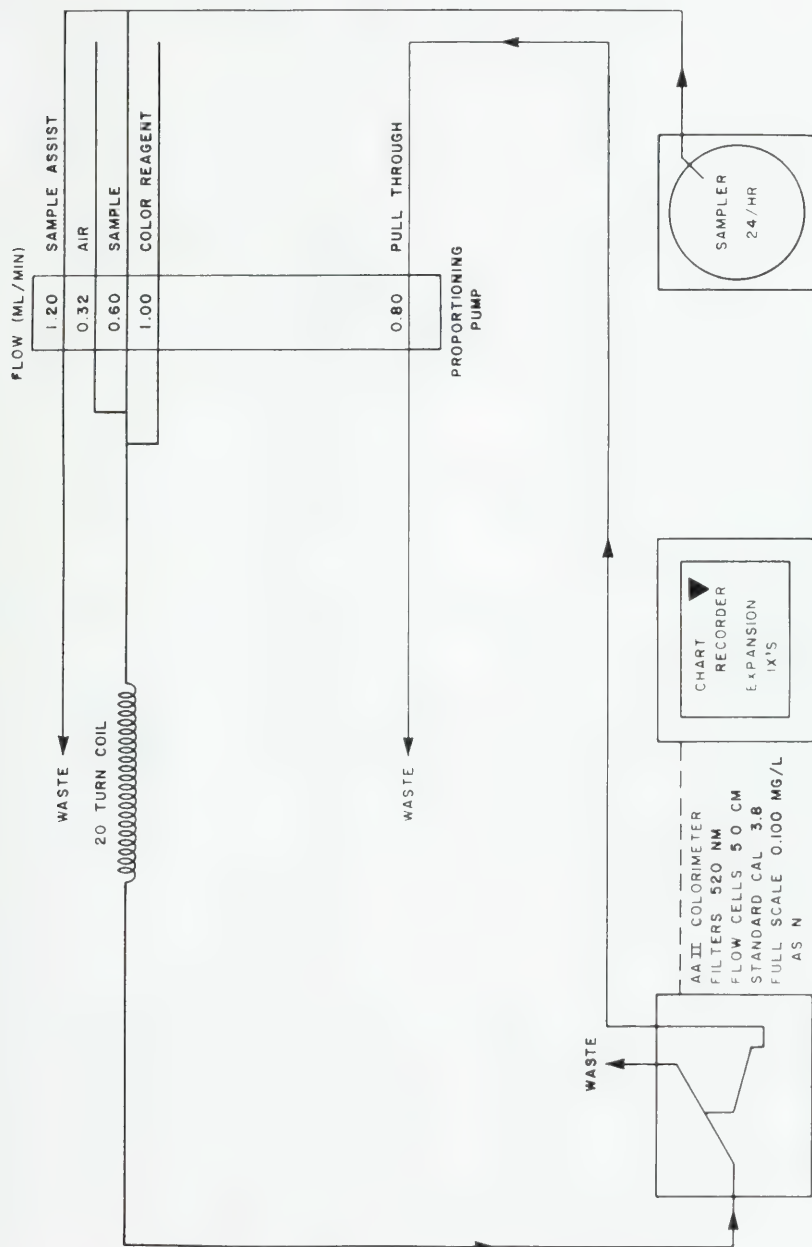


FIGURE 2 - AUTOMATED SYSTEM FOR FILTERED NITRITE NITROGEN, METHOD A.

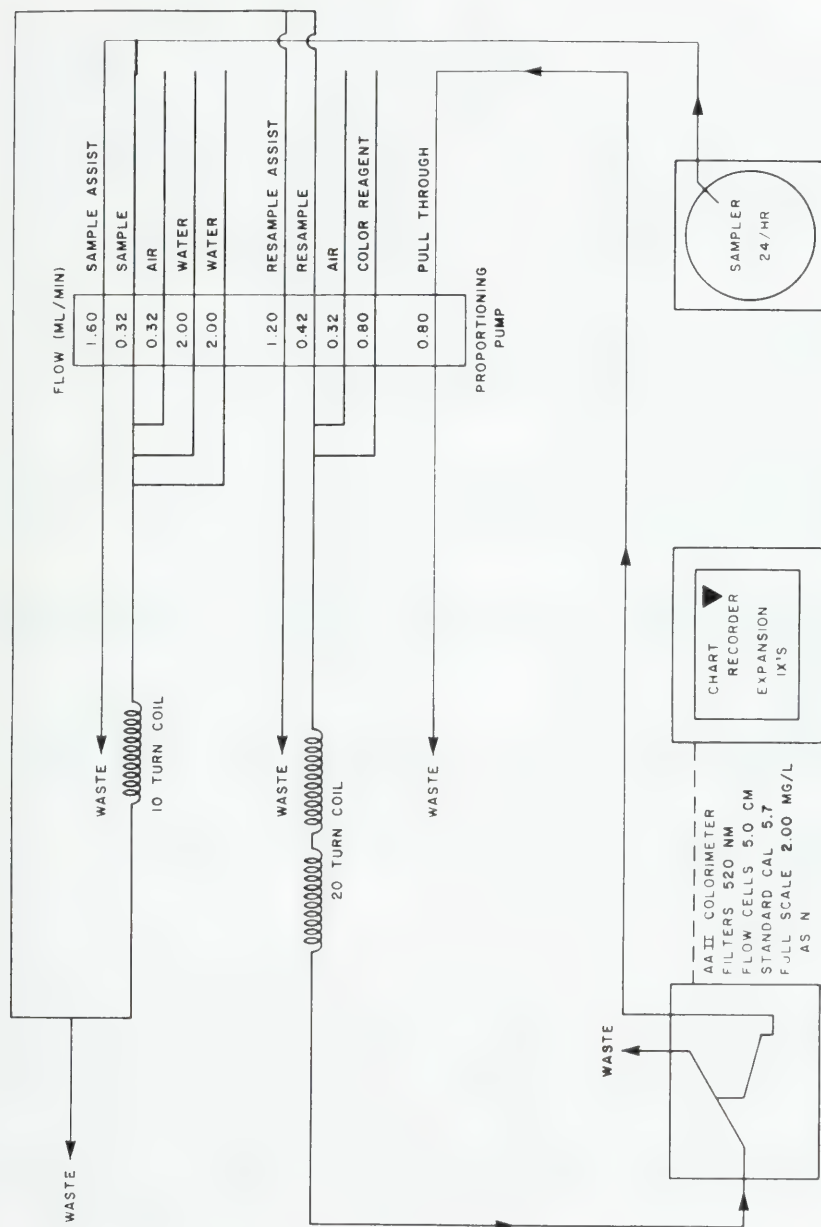
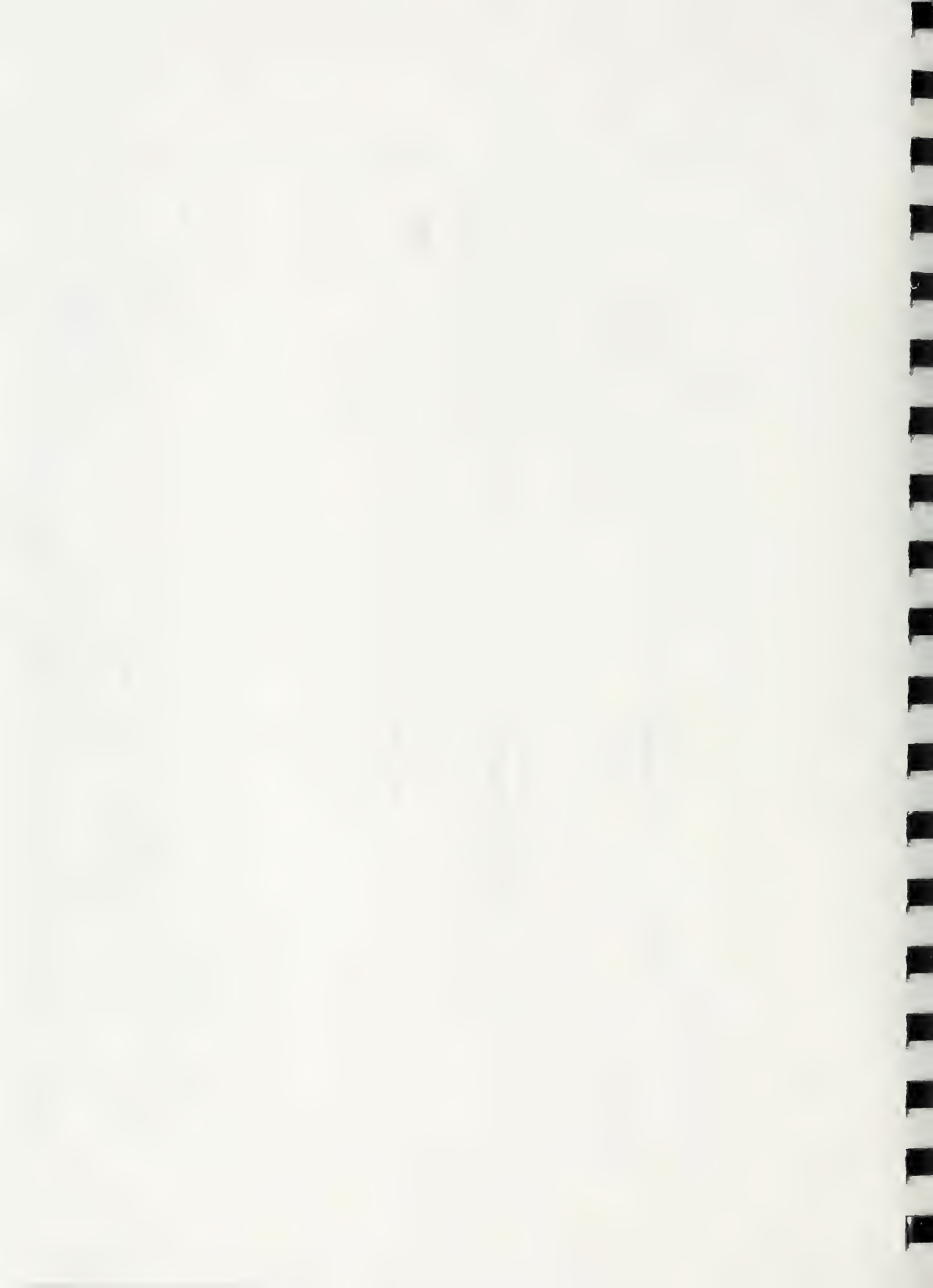


FIGURE 3 - AUTOMATED SYSTEM FOR NITRITE NITROGEN, VARIATION #1 OF METHOD A.



THE DETERMINATION OF TOTAL KJELDAHL NITROGEN

As nitrogen is one of the five major constituents of living matter, its form and concentration is of major interest when dealing with the growth of organisms in the aquatic environment. In nature, it may be found in a number of forms (organic nitrogen compounds, ammonia, nitrite, nitrate as well as nitrogen gas) all of which are interconvertible given the correct chemical and microbiological conditions. These species thus constitute the well-known "nitrogen cycle". Analytical methods have been developed to distinguish among them. Total Kjeldahl nitrogen (TKN) includes the organic nitrogen compounds (soluble and particulate) plus the ammonia fraction. If only organic nitrogen is of interest, it may be calculated by difference after an appropriate ammonia analysis has been completed (see the Determination of Ammonia Nitrogen).

Ammonia nitrogen is naturally present in surface, groundwaters, and wastewaters usually as the result of the de-amination of organic nitrogen compounds or by the hydrolysis of urea. Under anaerobic conditions reduction of nitrate to ammonia will also be important. Organic nitrogen compounds include soluble proteins, peptides, nucleic acids and urea as well as the nitrogen present in suspended organic particulates.

TKN concentrations may range from less than 0.1 mg/l as N in extremely "clean" environments to over 50 mg/l as N for wastewaters. Though not always the case, the organic nitrogen component of TKN usually constitutes the greater fraction for uncontaminated surface waters while the ammonia component is more significant in groundwater and wastewater samples.

Sewage and industrial waste treatment effluents and run-off from agricultural areas (where fertilizers have been used) are the primary sources of TKN beyond the natural nitrogen fixation and anaerobic nitrate reduction processes.

Sample Handling and Preservation

Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

At room temperature bacteria can convert organic nitrogen to ammonia, and ammonia to nitrite. These reactions are accelerated for domestic wastes due to the abundance of bacteria.

Selection of Method

Samples are analyzed for TKN via a semi-automated procedure that includes batch digestion, automated neutralization, and automated colorimetry. Aliquots of samples are digested, in a block digester, with Kjeldahl's reagent to convert the trinegative nitrogen content of organic compounds to ammonium ions (highly acidic media). Via an Auto-Analyzer system, the digested samples are neutralized and analyzed for ammonia species using phenate-hypochlorite colorimetry which determines both organic and ammonia forms of nitrogen. It should be noted that this digestion also serves to convert all phosphorus forms to orthophosphate and hence, total phosphorus analysis is simultaneously carried out on the same aliquot. Method A is used routinely for clean rivers and lakes, whereas variation #1 of Method A is used to analyse domestic water, sewage, leachates and industrial waste samples.

TOTAL KJELDAHL NITROGEN

Block Digestion - Automated Neutralization and Colorimetry Method A

SUMMARY

Matrix.	Method A is routinely used for clean rivers and lakes.
Substance determined.	Total Kjeldahl nitrogen (TKN) includes ammonia nitrogen plus organic nitrogen with trinegative valency. Both dissolved and particulate fractions of the sample are included in the analysis.
Interpretation of results.	Results are reported in mg/l as N. The digestion procedure converts organic nitrogen but not the nitrite or nitrate fractions to ammonia. TKN is of value in conjunction with total phosphorus and chlorophyll a values in assessing the state of enrichment in surface waters. High deviations from normal levels usually indicate the presence of a point source such as a sewage treatment plant outfall. Hypolimnetic water from eutrophic lakes may be high in dissolved ammonia and thus also high in TKN.
Principle of method.	TKN is determined as one of two semi-automated nutrient tests performed simultaneously on the same aliquot of sample. During digestion (batches of 40 samples), organic nitrogen is converted to ammonia in highly acidic media. After restoring the volume integrity, the sample is presented to an AutoAnalyzer system where it is neutralized in two stages, and then analyzed for ammonia species using phenate-hypochlorite colorimetry. The absorbance of the blue dye is measured at 630 nm. The TKN concentration is determined by comparison with a known set of standards.
Time required for analysis.	Samples are digested in batches of 40, and each batch requires two hours to process. For the AutoAnalyzer System, 24 analyses can be performed in one hour. Approximately 300 samples can be analyzed daily provided three block digesters are available.
Range of application.	0.01 - 2.00 mg/l as N on undiluted samples. Higher concentrations are determined by decreasing the volume of sample digested or by diluting the original sample.
Standard deviation.	The average standard deviation for duplicate analyses is 0.032 mg/l as N.
Accuracy.	The average recovery of control solutions is 99.8% with relative standard deviations ranging from 1.65 to 1.95%.

Detection criteria.	0.039 mg/l as N.
Interferences and shortcomings.	Due to high salt content, some samples must be diluted before analysis to avoid spattering during digestion. The average blank, which is associated with the digestion step, is 0.0165 mg/l as N.
Minimum volume of sample.	75 ml.
Preservation and sample container.	<p>Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.</p> <p>At room temperature bacteria can convert organic nitrogen to ammonia, and ammonia to nitrite. These reactions are accelerated for domestic wastes due to the abundance of bacteria.</p>
Safety considerations.	<p>Due to the use of concentrated sulphuric acid, considerable care is required during the digestion step, and particularly during the heating cycles. The latter should be conducted in a fume hood. Finally, arrangements must be made to dispose of the wastes from the test because they contain small amounts of mercury.</p>

NOTE: Phenol is an acid, highly toxic and readily absorbed through the skin. In case of contact, wash the affected area continuously with water and soap (or mild detergent) for at least five minutes.

TOTAL KJELDAHL NITROGEN

Block Digestion - Automated Neutralization and Colorimetry Method A

1. Introduction

Basically, this total Kjeldahl nitrogen (TKN) procedure entails converting the trinegative nitrogen content of organic compounds to ammonium ions in acid media, neutralizing, and then analyzing the sample for ammonia. Thus the TKN value also includes the original ammonia concentration. Both TKN and total phosphorus are determined on the same aliquot of sample, and Method A was designed for relatively clean rivers and lakes. Via Kjeldahl's reagent (sulphuric acid, potassium sulphate, and mercuric oxide) 40 samples are digested simultaneously in a two-step pretreatment; concentration at 200°C and fuming at 360°C for 20 minutes. The integrity of the sample volume is restored, and then the samples are presented to the AutoAnalyzer system where they are neutralized in two stages. The pH of the highly acidic samples (0.3 - 0.6 N) is raised to approximately 1.5 units by an alkaline-salt solution; the sodium chloride content complexes the mercuric ions in Kjeldahl's digestant. The samples are then buffered to pH 12.3 with a 1.0 M phosphate buffer and metal ions are complexed with EDTA. Finally, the sample is analyzed for ammonia nitrogen by phenate-hypochlorite colorimetry with sodium nitroprusside as catalyst. The absorbance of the blue indophenol species is measured at 630 nm using 1.5 cm flow cells. TKN results are determined from a chart recorder trace by comparison with peaks produced by undigested standards that contain appropriate quantities of the chemicals produced during the digestion step. Digested standards are utilized as recovery checks. (8.1, 8.2).

2. Interferences and Shortcomings

Due to high salt content, some samples must be diluted before analysis to avoid spattering during digestion. The average blank, which is associated with the digestion step, is 0.0165 mg/l as N. The potential interference from metals is avoided by the two stage neutralization procedure and the use of EDTA.

3. Apparatus

- 3.1. 3 BD-40 block digesters with temperature controllers.
- 3.2. Automated sampler.
- 3.3. Proportioning pump.
- 3.4. Heating bath (38°C) with 7.7 ml delay coil.
- 3.5. Colorimeter equipped with 630 nm filters and 1.5 cm flow cells.
- 3.6. Voltage regulator.
- 3.7. Chart recorder.
- 3.8. Pump tubing and associated glassware as indicated in Figure 1.

- 3.9. Digestion tubes, 2.5 x 20.0 cm, calibrated at 25 and 50 ml. Each tube is fitted with a 2.54 cm O-ring.
- 3.10. Digestion tube racks to fit block digester (8.2).
- 3.11. Vortex mixer.
- 3.12. Pipettors adjustable to predetermined volume.
- 3.13. Culture tubes, 1.8 x 15.0 cm.
- 3.14. Racks for culture tubes - 40 position.

4. Reagents

- 4.1. Ammonium chloride, NH_4Cl , reagent grade powder.
- 4.2. Aminoacetic acid (glycine), $\text{NH}_2\text{CH}_2\text{COOH}$, reagent grade crystals.
- 4.3. Sulphuric acid, H_2SO_4 , concentrated reagent grade.
- 4.4. Potassium sulphate, K_2SO_4 , reagent grade powder.
- 4.5. Mercuric oxide red, HgO , reagent grade crystals.
- 4.6. Anti-bumping granules, BDH#B33009.
- 4.7. Hydrochloric acid, HCl , concentrated reagent grade.
- 4.8. Sodium hydroxide, NaOH , reagent grade pellets.
- 4.9. Sodium chloride, NaCl , reagent grade crystals.
- 4.10. Potassium dihydrogen phosphate, KH_2PO_4 , anhydrous reagent grade powder.
- 4.11. Dipotassium hydrogen phosphate, K_2HPO_4 , reagent grade powder.
- 4.12. Phenol, $\text{C}_6\text{H}_5\text{OH}$, reagent grade crystals.
- 4.13. Sodium nitroferricyanide, dihydrate (sodium nitroprusside), $\text{Na}_2\text{Fe}(\text{CN})_5 \cdot 2\text{H}_2\text{O}$, reagent grade crystals.
- 4.14. Sodium hypochlorite solution, household bleach, 5.25% available chlorine.
- 4.15. Trisodium citrate, dihydrate, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, reagent grade crystals.
- 4.16. Ethylenediaminetetra-acetic acid, disodium salt, dihydrate (EDTA) $(\text{CH}_2\text{N} \cdot \text{CH}_2\text{COOH} \cdot \text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$, reagent grade crystals.
- 4.17. Potassium ferrocyanide, trihydrate, $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$, reagent grade crystals.

4.18. Stock Sulphuric Acid (20% v/v) Solution

Carefully add 1600 ml of concentrated sulphuric acid to 6400 ml of distilled, deionized water in increments while stirring and cooling in a sink of cold running water.

NOTE: Extreme care must be taken in handling sulphuric acid, a very corrosive chemical.

4.19. Hydrochloric Acid (10% v/v) Solution

Carefully add 100 ml of concentrated hydrochloric acid to 900 ml of distilled, deionized water. Prepare solution in a fume hood.

NOTE: Extreme care must be taken in handling hydrochloric acid, a very corrosive and pungent chemical.

4.20. Anti-Bumping Granules

Prepare anti-bumping granules by thoroughly washing them with hydrochloric acid (10% v/v), and then rinsing several times with distilled, deionized water. Dry granules overnight at 103°C.

4.21. Digestion Acid (Kjeldahl's Reagent)

Dissolve 10.0 g of mercuric oxide in 100 ml of stock sulphuric acid (20% v/v) solution. Carefully add 1 liter of concentrated sulphuric acid to 3 liters of distilled, deionized water while stirring. While the solution is still hot, add 670 g of potassium sulphate and dissolve. Add the mercuric oxide solution and 900 ml of distilled, deionized water. Mix well and store above 20°C.

4.22. pH Adjustment - Complexing Agent Solution

Dissolve 3.2 g of sodium hydroxide and 1.0 g of sodium chloride in 1 liter of distilled, deionized water.

4.23. Buffer

Dissolve 167 g of dipotassium hydrogen phosphate, 2.0 g of trisodium citrate and 18.6 g of the disodium salt of EDTA in approximately 600 ml of distilled, deionized water; add 40.4 g sodium hydroxide and dilute to one liter. Reagent is stable for at least one month. Reagent pH = 12.3.

4.24. Stock Phenol Solution

Melt 500 g of reagent grade phenol in its original container by warming in a water bath. Add 35 ml of distilled, deionized water to the liquidified phenol and allow to cool. Store this liquified phenol in the dark in a tightly stoppered bottle.

NOTE: Phenol is an acid, highly toxic and readily absorbed through the skin. In case of contact, wash the affected area continuously with water and soap (or mild detergent) for at least five minutes.

4.25. Phenate - Reducing Agent

Dissolve 4.0 g of potassium ferrocyanide and 25 g sodium hydroxide in about 800 ml of distilled, deionized water. Add 50 ml of stock phenol solution and dilute to 1 liter. Solution is unstable but can be used for three consecutive days. Reagent pH = 12.3.

4.26. Sodium Nitroferricyanide (0.4 g/l)

Dissolve 0.4 g of sodium nitroferricyanide (commonly called sodium nitroprusside) in one liter of distilled, deionized water. Solution can be used for three consecutive days. Reagent pH \approx 6.5, i.e., very similar to the pH of the day's water supply.

4.27. Hypochlorite Solution

Dilute 70 ml of sodium hypochlorite solution (household bleach) to 1 liter with distilled, deionized water.

4.28. Wash Water

To maintain a suitable baseline for the AutoAnalyzer system, prepare wash water by diluting 300 ml of stock sulphuric acid (20% v/v) solution to 4 liters with distilled, deionized water and dissolving 53.6 g of potassium sulphate.

4.29. Concentrated Stock Standard TKN Solution

Dissolve 3.8189 g ammonium chloride in 1 liter of distilled, deionized water. Concentration with respect to TKN is 1000 mg/l as N.

4.30. Intermediate Stock Standard TKN Solution

Dilute 40.0 ml of the concentrated stock standard TKN solution to 1 liter with distilled, deionized water. Concentration with respect to TKN is 40 mg/l as N. This standard may be used for one working week.

NOTE: In actual practice this intermediate stock standard also contains orthophosphate: 4.00 mg/l as P.

4.31. Working Calibration Standards

Two working standards are prepared daily from the intermediate stock standard:

Low (L): dilute 5.00 ml of intermediate stock to 1 liter with wash water. Concentration with respect to TKN is 0.200 mg/l as N.

High (H): dilute 40.0 ml of intermediate stock to 1 liter with wash water. Concentration with respect to TKN is 1.60 mg/l as N.

Blanks (BI): wash water.

4.32. Super Stock TKN Recovery Standard Solution

Dissolve 15.0050 g aminoacetic acid (glycine) in 1 liter of distilled, deionized water. TKN concentration is 2800 mg/l as N.

4.33. Stock TKN Recovery Standard Solution

Dilute 100.0 ml of super recovery stock to 1 liter with distilled, deionized water. TKN concentration is 280 mg/l as N.

NOTE: In actual practice the stock recovery standard also contains pyrophosphate: 28.0 mg/l as P.

4.34. Intermediate Stock TKN Recovery Standard Solution

Dilute 10.0 ml of the stock TKN recovery standard to 2 liters with distilled, deionized water. TKN concentration is 1.40 mg/l as N.

4.35. Working TKN Recovery Standards

Three levels of the intermediate stock TKN recovery standard and distilled, deionized water are analyzed daily:

Solution No.	Volume of Standard (ml)	Final Volume (ml)	TKN Concentration (mg/l as N)
R ₁	25.0	25.0	1.40
R ₂	15.0	25.0	0.84
R ₃	5.00	25.0	0.28
DB*	25.0	25.0	0

*The digested blank (DB) is distilled, deionized water.

4.36. Quality Control Solutions

The concentrations of the QC solutions are chosen such that they cover the normal concentration range of samples being routinely analyzed. Sufficient volumes are prepared to last a minimum of 20 days of analyses; whenever new QC solutions are required, they are prepared in advance so that they may be monitored for at least three days prior to adopting them.

For TKN, quality control (QC) solutions are prepared by diluting a concentrated stock solution of ammonium chloride with wash water. A long term blank of the appropriate batch of wash water is analyzed daily.

5. Procedure

- 5.1. Collect the samples and group them according to the bench sheet.
- 5.2. Transfer 25.0 ml of sample via a wide mouth pipet to a digestion tube. Smaller aliquots may be selected if the TKN concentration is known to exceed 1.9 mg/l as N.
- 5.3. To the digestion tube, add 4.0 ml of digestion acid by a precalibrated pipettor and a few anti-bumping granules.
- 5.4. Mix the sample on a Vortex mixer to ensure homogeneity and place the tubes in a digestion tube rack maintaining sequential order.
- 5.5. Place the rack of treated samples in the DB-40 block digester which is maintained at 200°C.
- 5.6. When the water from the samples has evaporated (1 - 1.5 hour), transfer the rack to the DB-40 block digester which is maintained at 360°C. Leave the samples in this block digester for 20 ± 1 minutes, and then remove.

- 5.7. After cooling add 25.0 ml distilled, deionized water to each tube, and use a vortex mixer to thoroughly mix the contents. If carbon particles are visible, discard the solution, and repeat the analysis at a higher factor.
- 5.8. Transfer the entire digested sample to a clean dry culture tube, and place in culture tube racks. Number the first and last tube of each series of ten samples in accordance with the bench sheets.
- 5.9. Set the AutoAnalyzer system in operation using cleaning and checking procedures appropriate to the manifold illustrated in Figure 1.
- 5.10. When loading the samples into the sampler module, ensure that the sample order conforms to the bench sheet.
- 5.11. Each run of samples will include all of the following units:

Set of undigested calibration standards: H, L
 Blank (day's supply of wash water): Bl
 Quality Control (QC) samples: QC-A, QC-B
 Long term blank: LT Bl
 Recovery Standards: R₁, R₂, R₃
 3 Digested Blanks: DB

The basic sample loading sequence is:

10 samples, Bl, 10 samples, L, H, Bl.

- 5.12. Calibrate the AutoAnalyzer system using calibration standards. Record the standard calibration setting, and check that it has not changed unduly.
- 5.13. Confirm the calibration by analyzing the quality control solutions. Record these values. For each analytical range, calculate totals and differences, i.e., QC-A plus QC-B and QC-A minus QC-B. Check to ensure that the calculated values conform to limits based on past data.
- 5.14. Analyze the recovery standards and digested blanks. Check to ensure that all values conform to limits based on past data. If this condition is not met, do not proceed until the situation has been resolved. The recovery checks monitor both the digestion step and the AutoAnalyzer system.
- 5.15. Monitor calibration standards (in-run sensitivity checks) throughout the run to determine if corrections are required.
- 5.16. Read sample peak heights, and convert to concentration values.

6. Calculation and Reporting

If the aliquot of sample ranged from 5.00 to 25.0 ml, multiply the chart reading by the factor:

$$\frac{25.0}{\text{aliquot volume (ml)}}$$

If a dilution of the sample was prepared, and 25.0 ml taken for analyses, multiply the chart reading by the dilution factor:

$$\frac{\text{diluted volume}}{\text{aliquot volume}}$$

In both cases, record the result in the answer space opposite each sample number of the bench sheet. Report the results to three significant figures if feasible.

7. Precision and Accuracy

Precision - duplicate analyses of routine samples

Sample Concentration Range (mg/l as N)	Standard Deviation (mg/l as N)
<0.40	0.027
0.40 - 1.00	0.036
1.00 - 2.00	0.039

Accuracy-recovery of quality control (QC) solutions

QC Concentration (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
0.50	0.493	0.0065
1.50	1.501	0.0134

Accuracy - analyses of recovery standards

Concentration of Recovery Standard (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
0.84	0.840	0.0138
1.40	1.393	0.0274

8. Bibliography

- 8.1. Crowther, J., Wright, B., and Wright, W., "Semiautomated Determination of Total Phosphorus and Total Kjeldahl Nitrogen In Surface Waters", Anal Chim Acta 119, 313, 1980.
- 8.2. Crowther, J., Wright, B., and Wright, W., "Semi-Automated Procedure For The Determination of Total Nutrients", Internal Report For Project JC7801, dated May 28, 1979.

TOTAL KJELDAHL NITROGEN

Block Digestion - Automated Neutralization and Colorimetry Method A

Variation #1

SUMMARY

Matrix.	Domestic water, sewage, leachates, industrial waste.
Substance determined.	TKN includes ammonia nitrogen plus organic nitrogen with trinegative valency. Both dissolved and particulate fractions of the sample are included in the analysis.
Interpretation of results.	Results are reported in mg/l as N. The digestion procedure converts organic nitrogen but not the nitrite or nitrate ions to ammonia. TKN is of value in estimating the loadings to waste treatment facilities and in assessing the efficiency of such plants. The TKN concentration in treated effluents is a measure of the potential burden placed on the receiving water.
Principle of method.	TKN is determined as one of two semi-automated nutrient tests performed simultaneously on the same aliquot of sample. During digestion (batches of 40 samples), organic nitrogen is converted to ammonia in highly acidic media. After restoring the volume integrity, the sample is presented to an AutoAnalyzer system where it is neutralized by dilution, and then analyzed for ammonia species using phenate-hypochlorite colorimetry. The absorbance of the blue dye is measured at 630 nm. The TKN concentration is determined by comparison with a known set of standards.
Time required for analysis.	Samples are digested in batches of 40, and each batch requires two hours to process. For the AutoAnalyzer system, 24 analyses can be performed in one hour. Approximately 300 samples can be analyzed daily provided three block digesters are available.
Range of application.	0.1 - 10.00 mg/l as N. Higher concentrations are determined by decreasing the volume of sample digested or by diluting the original sample.
Standard deviation.	The average standard deviation for duplicate analyses is 0.072 mg/l as N.
Accuracy.	The average recovery of control solutions is 100.7% with relative standard deviations ranging from 1.86 to 4.01%.
Detection criteria.	0.087 mg/l as N.

Interferences and shortcomings.

Due to high salt content, some samples must be diluted before analysis to avoid spattering during digestion.

Minimum volume of sample.

75 ml.

Preservation and sample container.

Glass or polystyrene containers are acceptable. To obtain reliable results, samples should be refrigerated and shipped to the laboratory as rapidly as possible. Samples may be frozen.

At room temperature bacteria can convert organic nitrogen to ammonia, and ammonia to nitrite. These reactions are accelerated for domestic wastes due to the abundance of bacteria.

Safety considerations.

Due to the use of concentrated sulphuric acid, considerable care is required during the digestion step, and particularly during the heating cycles. The latter should be conducted in a fume hood. The alkaline phenate reagent is extremely corrosive as is the liquified phenol and sodium hydroxide used to prepare it. Phenol and phenate spills should be immediately rinsed with methanol (keep away from eyes) followed by copious quantities of cold water. Finally, arrangements must be made to dispose of the wastes from the test because they contain small amounts of mercury.

TOTAL KJELDAHL NITROGEN

Block Digestion - Automated Neutralization and Colorimetry Method A

Variation #1

1. Introduction

The total Kjeldahl nitrogen (TKN) contents of domestic water supplies, sewage, industrial wastes and leachates are determined by a less sensitive variation of Method A. The changes entail higher acid digestant concentrations, and replacement of the first neutralization step by a dilution loop.

2. Interferences and Shortcomings

Due to high salt content, some samples must be diluted before analysis to avoid spattering during digestion. The potential interference from metals is avoided by neutralization, dilution and the use of EDTA.

3. Apparatus (Changes)

- 3.1. Colorimeter is equipped with 5.0 cm flow cells instead of 1.5 cm flow cells.
- 3.2. Pump tubing and associated glassware are modified as indicated in Figure 2.

4. Reagents (Changes)

- 4.1. Trisodium citrate dihydrate, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, reagent grade crystals is not required.
- 4.2. The pH adjustment - complexing agent solution is prepared by dissolving 0.9 g of sodium chloride in 1 liter of distilled, deionized water (The adjustment of pH to an intermediate stage is accomplished by dilution.)
- 4.3. The buffer is prepared by dissolving 87 g of dipotassium hydrogen phosphate and 18.6 g of the disodium salt of EDTA in approximately 600 ml of distilled, deionized water; add 18.5 g of sodium hydroxide and dilute to 1 liter with distilled, deionized water. Reagent is stable for at least one month. Reagent pH = 11.4 to 12.0.
- 4.4. The phenate - reducing agent is prepared by dissolving 4.0 g of potassium ferrocyanide and 20 g of sodium hydroxide in about 800 ml of distilled, deionized water. Add 50 ml of stock phenol solution and dilute to 1 liter with distilled, deionized water. Solution is unstable but may be used for three consecutive days. Reagent pH = 11.8.

NOTE: Extreme care must be taken in handling sodium hydroxide, a very caustic chemical.

- 4.5. To maintain a suitable baseline for the AutoAnalyzer system, the wash water is prepared by diluting 320 ml of stock sulphuric acid (20% v/v) solution to approximately 4.5 liters; add 48 g of potassium sulphate, dissolve, and mix thoroughly.
- 4.6. Calibration standards contain both TKN and phosphorus, but are prepared by diluting stock solutions of the individual components. The diluent for the working standards (undigested) is the foregoing wash water (4.5). The working TKN concentrations are 8.00 and 2.00 mg/l as N.

- 4.7. Recovery standards (digested) contain both aminoacetic acid (glycine) and tetrasodium pyrophosphate, and are prepared by diluting stock solutions of the individual components with distilled, deionized water. The working TKN concentrations are 7.00 and 1.40 mg/l as N.
- 4.8. Quality control solutions (undigested) for monitoring AutoAnalyzer calibration are prepared by diluting with wash water a third set of TKN and phosphorus stock solutions.

5. Procedure

- 5.1. For the digestion step, the selected aliquot of sample ranges from 5.0 to 25.0 ml. The volume of acid digestant is increased to 4.0 ml. The final volume of digested sample is always 50.0 ml. In effect, the volume of the acid digestant is increased and the dilution factor is always two or more.
- 5.2. Analyses are completed in accordance with standard procedures for Auto-Analyzer (see Method A and Figure 2).

6. Calculation and Reporting

Multiply the reading by the dilution factor

$$\frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result in the answer space opposite each sample number of the bench sheet. Report the results to three significant figures.

7. Precision and Accuracy

Precision - duplicate analyses of routine samples

Sample Concentration Range (mg/l as N)	Standard Deviation (mg/l as N)
<2.00	0.053
2.00 - 5.00	0.079
5.00 - 10.0	0.088

Accuracy-recovery of quality control (QC) solutions

QC Concentration (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
1.40	1.420	0.0297
7.00	7.033	0.0862

Accuracy - analyses of recovery standards

Concentration of Recovery Standard (mg/l as N)	Average Recovery (mg/l as N)	Standard Deviation (mg/l as N)
1.40	1.431	0.0562
7.00	6.935	0.1299

8. Bibliography

- 8.1. Crowther, J., Wright, B., and Wright, W., "Semiautomated Determination of Total Phosphorus and Total Kjeldahl Nitrogen In Surface Waters", Anal Chim Acta 119, 313, 1980.
- 8.2. Crowther, J., Wright, B., and Wright, W., "Semi-Automated Procedure For The Determination of Total Nutrients", Internal Report For Project JC7801, dated May 28, 1979.

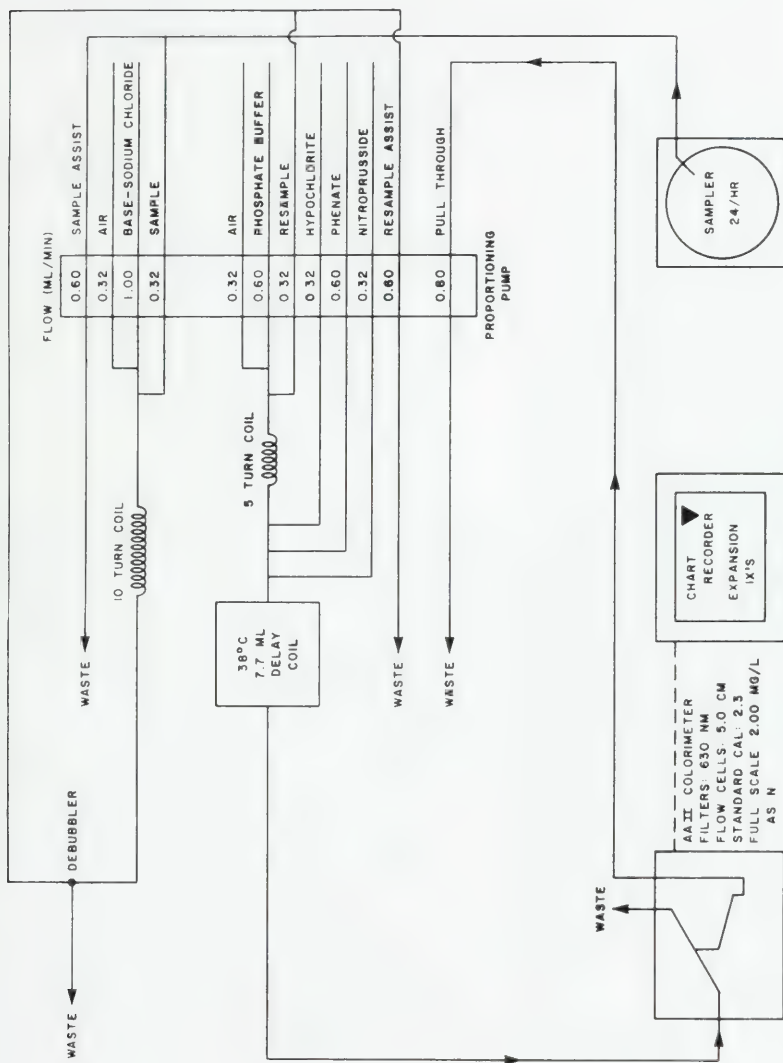


FIGURE 1 - AUTOMATED NEUTRALIZATION - COLORIMETRIC SYSTEM FOR TOTAL KJELDAHL NITROGEN, METHOD A.

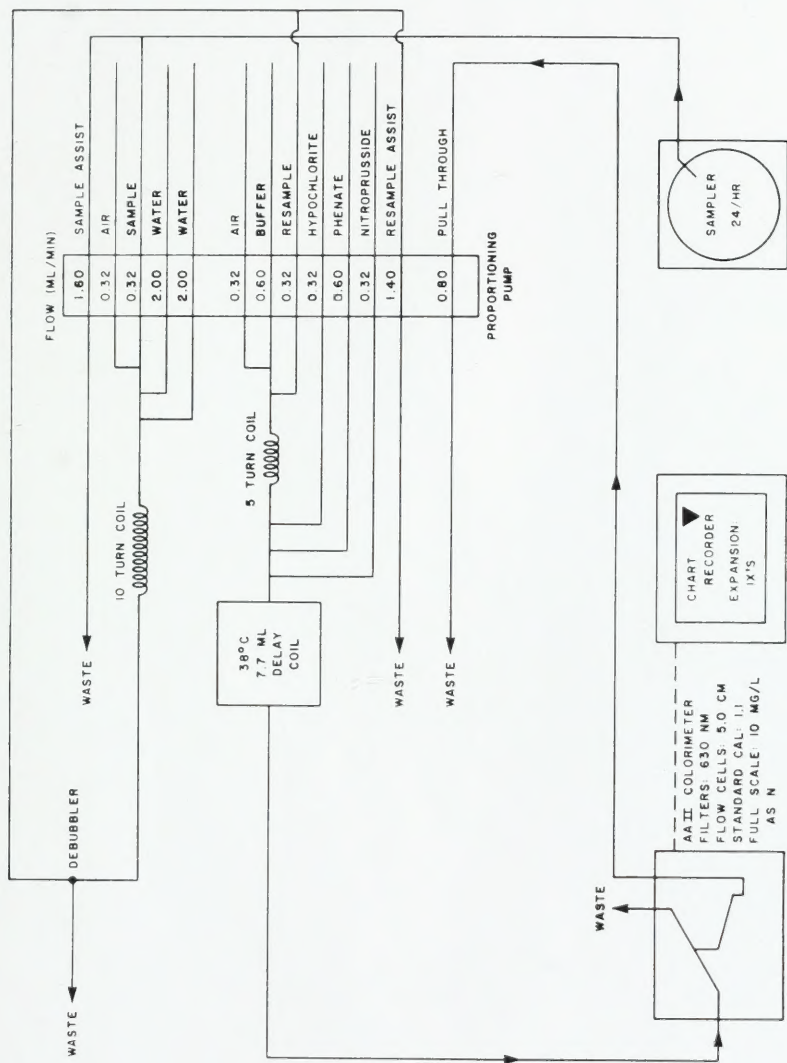


FIGURE 2 - AUTOMATED NEUTRALIZATION - COLORIMETRIC SYSTEM FOR TOTAL KJELDAHL NITROGEN, VARIATION #1 OF METHOD A.

Figure 1. Schematic diagram of the experimental setup.





